A NATIONAL UNDERSTANDING FOR THE DEVELOPMENT OF REFERENCE MATERIALS AND METHODS FOR CLINICAL CHEMISTRY

Proceedings of a Conference

Edited by:
Joseph H. Boutwell, M.D., Ph.D.

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Proceedings of a Conference

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"Will you walk a little faster?" said a whiting
to a snail,
"There's a porpoise close behind us, and he's
treading on my tail."

. . . . . .

Will you, won't you, will you, won't you,
won't you join the dance?

". . . Don't you mean 'purpose'?" said Alice.

—Lewis Carroll
(Alice's Adventures in Wonderland)

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A Short History of the Conference [Ed.]

The Conference was sponsored by the Center for Disease Control (CDC), the Food and Drug Administration (FDA), and the National Bureau of Standards (NBS). The three agencies have long been deeply involved in the development of reference materials and methods as well as in the voluntary consensus process for laboratory standards. Discussions (as early as 1974-5) among the three agencies led to the conclusion that the relationships involved in the existing system(s) for the development of reference methods and materials were extremely complex (and were becoming more complex) and were highly interactive with the private sector(s). The government group (CDC, FDA, NBS) became convinced that only by some sort of national "meeting of minds" could the government agencies properly manage their own contributions to the developing national reference system.

An initial plan for a meeting in late 1976 proved too ambitious and it was not until July of 1976 that initial program proposals were circulated to proposed co-sponsors for comment. On September 1, 1976, representatives of the three government agencies, industry, and professional associations were convened in Atlanta at the Center for Disease Control. This planning meeting was chaired by Dr. David D. Bayse of CDC with the following attendees:

Dr. John Batsakis - CAP/ASCP
Dr. David D. Bayse - CDC
Dr. Joseph Boutwell - CDC
Dr. Stanley Brown - England (at that time WHO Consultant to CDC)
Dr. Eloise Eavenson - FDA
Mr. Jerry Gallwas - Industry
Dr. Pierre Keitges - NCCLS
Dr. Alan Mather - CDC
Dr. Richard Nadeau - NCCLS
Dr. Royden Rand - AACC
Dr. John Ross - CAP/ASCP
Dr. Robert Schaffer - NBS
Dr. Raymond Vanderlinde - AACC

The tentative agenda for this meeting was as follows:

September 1, 1976

TENTATIVE AGENDA

I. A PROPOSAL FOR A "CONFERENCE ON A NATIONAL UNDERSTANDING FOR THE DEVELOPMENT OF REFERENCE METHODS AND MATERIALS FOR CLINICAL CHEMISTRY"

There is a definite need for highly coordinated national planning in the area of the development of reference method systems in clinical chemistry. The concept of reference method systems would embrace reference methods, reference materials, and definitive methods, as well as the operational application of these components. This planning would develop a common basis to guide scientific efforts of the Nation's clinical chemistry investigators in the development of these reference method systems.

In order to obtain a broad base of advice and support for such an important undertaking, the involved Government agencies and private professional organizations (including industry) will develop a series of position papers on reference method systems for review by the Nation's expert clinical chemistry scientists, and sponsor a joint conference to critique these papers in the Fall of 1977.

The Center for Disease Control (CDC) will host and coordinate this Conference. The program content will consist of individual and/or collective opinions of the sponsoring organizations. The final version of the position papers, the important deliberations of the Conference, and significant statements from each of the Presidents or designated representatives of each of the sponsoring organizations will be published by the American Association for Clinical Chemistry (AACC). [Note: A request to this effect was forwarded to AACC at this time.]

The above proposal has been developed in concert with Dr. Robert Schaffer of the National Bureau of Standards (NBS) and Dr. Eloise Eavenson of the Food and Drug Administration (FDA), and has been reviewed in a series of discussions with other important national leaders in clinical chemistry.

II. BRIEF DISCUSSION OF THE AGENDA AND REACTION TO THE PROPOSAL

III. DISCUSSION OF THE (PROPOSED) CONTENT OF THE POSITION PAPERS FOR THE CONFERENCE

IV. PLAN FOR PREPARATION AND ASSIGNMENT OF RESPONSIBILITY FOR THE POSITION PAPERS

V. DEVELOPMENT OF LIST OF ATTENDEES FOR THE NATIONAL CONFERENCE

VI. MEETING FORMAT

A. The proposed meeting would have a format of position papers circulated to attendees prior to the meeting.

B. At the time of the Conference, sponsoring organizations would critique the position papers.

C. Discussion groups would be formed and would report back to the Conference their oral and written opinions.

VII. PUBLICATION OF THE POSITION PAPERS AND PROCEEDINGS OF THE CONFERENCE

VIII. COORDINATION AND SITE OF THE CONFERENCE

The objective of the Conference as determined by this group was to provide an opportunity for a full, free, and open discussion of the issues surrounding reference methods development, acceptance, and use in order to arrive at a national understanding of the roles played by all concerned agencies, institutions, and individuals.

The framework for the Conference was developed in the discussions by the Steering Committee and was not materially modified thereafter. The final plan became quite simple: (1) Sponsors and co-sponsors would develop organizational "position papers," which would be distributed to participants and observers prior to the Conference. (2) The Conference would consist of a critique and discussion of the position papers and, (3) the development of statements and recommendations reflecting the understanding developed by the Conference. (4) Publication of the Proceedings and the Recommendations.

Invitations to the proposed organizational participants were quickly accepted and Dr. David D. Bayse, as Conference Chairman, immediately started to develop the specific details and guidelines for the program and to arrange for the facilities for the Conference which by November 1976, had been scheduled for lage Fall 1977.

The final Steering Committee consisted of institutional representatives as follows:

AACC	-	Royden Rand Ray Vanderlinde	FDA	-	Eloise Eavenson
			NBS	-	Philip LaFleur
ASCP	-	Thorne Butler			Robert Schaffer
CAP	-	John Batsakis John Ross	NCCLS	-	Pierre Keitges Richard Nadeau
CDC	-	David Bayse Joseph Boutwell	INDUSTRY	-	Jerry Gallwas

POST CONFERENCE DEVELOPMENTS [Ed.]

The Conference developed a significant consensus for the development of a proposal by NCCLS for the management of a National Reference System for Clinical Chemistry. NCCLS was requested to develop a proposed plan and to submit it to participants and observers (both institutional and individual) for comment and criticism.

NCCLS has developed a proposal which has been circulated for comment. It was presented to the NCCLS constituency at its annual meeting (April 3-5, 1978) and to the National Committee on Health Laboratory Services (NCHLS)(April 15, 1978). It was also presented to the Steering Committee at its final meeting in Atlanta on May 2, 1978. Further revisions and modifications of the proposal are expected as a result of comments received from the laboratory community.

It has been widely recognized that while the organizational and management skills of NCCLS are extremely important, the further development of this national reference system process and procedure and also its effectiveness will really depend upon the operational consensus acceptance by all of the institutions and individuals participating in and contributing to this process.

Editor's Note:

These Proceedings consist, except for certain very obvious editorial additions, of the texts of material received from the sponsors or co-sponsors, from the Working Group Chairmen, or from the individual indicated. The formats have been modified to maximize space utilization, and minimal grammatic and orthographic changes have been made. No attempt was made to achieve consistency of terminology or of concept, because of the nature of the Conference and prior commitments made to the participants.

Conference

on a

National Understanding

for the development of

Reference Methods and Materials

for

Clinical Chemistry

November 16-17, 1977

Atlanta, Georgia

Sponsors

Center for Disease Control
Food and Drug Administration
National Bureau of Standards

Co-sponsors

American Association for Clinical Chemistry
American Society of Clinical Pathologists
College of American Pathologists
Health Industry Manufacturers Association
National Committee on Clinical Laboratory Standards
Pharmaceutical Manufacturers Association
Scientific Apparatus Makers Association

Conference Chairman: Dr. David D. Bayse - CDC

Conference Coordinator: Dr. Gary Myers - CDC

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CAP	Dr. Roger K. Gilbert
CDC	Dr. David D. Bayse, Chairman
FDA	Dr. Eloise Eavenson
INDUSTRY	Mr. Gerald Gallwas
NBS	Dr. Philip D. LaFleur
NCCLS	Dr. Pierre Keitges

FORMAT

The Conference is planned as a two-day meeting for approximately 150-200 participants representing the concerned Federal Agencies, clinical laboratory professions, and the appropriate health industries.

The Conference will address three major topics, each of which will be subdivided into more manageable working groups. Sponsors and co-sponsors will have a designated representative on each Working Group. The purpose of these Working Groups will be to review the scientific advice contained in position papers submitted prior to the Conference by the sponsors and co-sponsors. This advice will be reviewed, and, together with the deliberations of the Working Group, will be organized into the Proceedings of the Conference which will be published at an early date.

OBJECTIVES

This Conference will attempt to further the understanding of the components necessary for the development, the acceptance, and the utilization of reference materials and methods in clinical chemistry.

Such a scheme on a national basis can be termed a National Reference System in Clinical Chemistry (NRSCC).

The objectives of this two-day Conference are (1) to define criteria for and essential characteristics of reference methods and materials used in establishing national standards for the accuracy of quantitative methods in clinical chemistry, and (2) to develop a total framework in which government agencies, professional organizations, and industry can cooperate on a national basis in the orderly development of a National Reference System in Clinical Chemistry.

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AMERICAN ASSOCIATION FOR CLINICAL CHEMISTRY

POSITION PAPER

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Position of the American Association for Clinical Chemistry with regard to Reference Methods and Materials

GENERAL STATEMENT

The purpose of the American Association for Clinical Chemistry (AACC), stated in its Constitution, is "to further the public interest by encouraging the study, advancing the science and improving the practice of clinical chemistry". It has long been a primary mission of AACC to seek true values in clinical chemistry, and thus to provide the basis for results which are compatible from laboratory to laboratory and from time to time. Inadequate accuracy and inadequate precision lead to inadequate patient care.

AACC was an early supporter of the concept of reference methods and reference materials, and will continue to participate willingly and actively in the development of a national reference system for clinical chemistry. The strength of AACC lies in its reservoir of members with analytical skills and professional concern. AACC, through its members, assumes an active, responsible role in improving the performance of individual laboratories, and takes an initiating role in groups seeking to improve the reliability and clinical usefulness of laboratory findings. In this respect its aims are:

(1) to support the criterion of accuracy in the documentation of laboratory performance

(2) to study the specificity, sensitivity and predictive value of diagnostic laboratory procedures

(3) to discover areas where improved analytical performance is needed

(4) to formulate principles and define conditions for improvement of procedures, and to explore new areas of potential diagnostic interest

(5) to identify, investigate and validate various levels of reference methods and materials

(6) to study reference ranges pertinent to individuals and

to populations in defined states of health or disease

AACC activities will be coordinated through its national organizational structure.

ORGANIZATIONAL COMPONENT

During the last decade complex and multi-faceted programs have emerged to cope with the need for improved accuracy in clinical chemistry measurements. Professional associations, such as the American Association for Clinical Chemistry, have stimulated the research and publication of new techniques and the participation of their memberships in Committee efforts and as consultants to government agencies. Private, state and federal programs have been instituted for the evaluation of clinical laboratory proficiency by comparison of performance with peer groups and reference laboratories.

The National Bureau of Standards has provided Clinical Reference Materials for clinical chemistry and, with financial incentives from the Department of Health, Education, and Welfare as well as internal funds, has turned its considerable analytical talents to the development of highly accurate definitive methods for this field. The Center for Disease Control committed resources to the development of reference methods for frequently determined clinical constituents.

Implementation of new legislation regarding the control of manufactured diagnostic products for use in the clinical laboratory has led to several programs by the Food and Drug Administration. In particular, the FDA efforts to prepare accuracy criteria for the regulated diagnostic products, created a strong stimulus for more rapid development of analytical yardsticks applicable to clinical chemistry measurments.

Coordination and integration of the above private and governmental programs has been based on numerous personal and professional relationships, interagency memoranda of understanding and various ad hoc cooperative undertakings. The time appears to be at hand for a logical, centralized coordination of this expanding activity which gives adequate

recognition to all parties concerned with clinical chemistry measurements. These needs are concurrent with with the public concern over the rapidly rising cost of health care (estimated at $115 billion for 1977). Therefore, any new programs must take maximum use of existing resources and be concerned with cost effectiveness.

ANALYTICAL GOALS AND QUALITY REQUIREMENTS

In concert with other biomedical scientists and many physicians (1), the clinical chemist tends to think of human health and illness in terms of functional or molecular disorders (2). As biochemical researchers continue to discover the importance of measuring body fluid concentrations and/or turnover rates of key chemical constituents in the blood serum or other fluid and tissue compartments which reflect changes at the molecular level, the demand for quantitative chemical measurements in clinical medicine has increased dramatically (3).

RESPONSE TO ANALYTIC NEEDS

In response to these needs for quantitative measurements, a variety of new analytical methods and instruments, kits and human skills have been brought into clinical chemistry service laboratories over the past two decades (4). The initial criteria for the acceptance of these new scientific and technical resources were availability and repeatability. Since there were few accepted reference materials and no reference methods, questions about specificity, systematic bias and interferences, even if asked, were not easily answered. Thus, when each individual clinical laboratory scientist or technologist made his own choice of method, which frequently was inflexibly embodied in an instrumental system or in a kit with secret ingredients, and since the number of methodologic variables is large, it should come as no surprise that results between laboratories were often markedly different. For example in clinical enzymology, where reference materials and reference methods for enzymes were and are still lacking, the results for enzyme tests show particularly obvious and confusingly large incompatibilities (5).

COMPATIBILITY BASED UPON TRUE VALUE

It is quite clear that analytical results from clinical chemistry laboratories must be made compatible. Measurement systems based upon making accurate measurements, i.e., measurements directly related or traceable to the "true value" of the property(ies) under test, have been demonstrated to be compatible (6-9). To the individual chemist, this means that results should agree from laboratory to laboratory and over time because an accurate method yields "true value". To the chemists collectively this means that the fundamental components and interrelationships needed to achieve accuracy must be built into a measurement system. This system must at the very least rest upon objective agreements concerning high-purity, well characterized refrerence materials and carefully studied methods of known accuracy. As practicing clinical chemists who are daily involved in the quantitative chemical measurement process of patient samples, wholeheartedly agree that ACCURACY is "an idea whose time has come'(10).

ADDITIONAL CRITERIA

If we are to avoid unproductive debate and misunderstanding in attempts to develop analytical goals and quality requirements for a national reference system for clinical chemistry, each of you must recognize the unique contributions that the other can and must bring to these discussions. We endorse the importance of "medical usefulness" as one basis for our discussions; however, insights which equally as valid can be gained from at least two additional concepts that have not been heretofore specifically delineated. These deal with consideration of a) molecular biology and b) analytical chemistry.

It is our opinion that analytical goals and quality requirements be discussed and resolved more fully if we have properly:

1) identified the biochemical pathways and critical analyte(s) which directly affect the cell (a molecular biology question).

2) assured ourselves that the methods to measure the

concentration or other property of these analytes are accurate (an analytical chemistry question).

3) established the clinical sensitivity and specificity of the analyte(s) in the diagnosis and treatment of well characterized disorders (a medical usefulness question).

The following statements on calcium may help to illustrate the need for these three distinct although admittedly interrelated terms of reference:

Calcium is recognized as an essential element in biological systems and it is the unbound calcium (free or ionic -- Ca^{2+}) which is the critical molecular species (11). However, total serum calcium when corrected for pH and albumin influences reflects the changes in unbound calcium (12) (a statement in terms of molecular biology).

Total calcium can be measured very accurately in serum (13,14) but there is as yet continued disagreement and little widespread daily service experience with analytical systems of clearly defined accuracy for measuring ionic calcium (15-17) (a statement in terms of analytical chemistry).

The clinical sensitivity and specificity of total calcium concentrations in serum for some disorders of calcium metabolism are well established. In contrast, the usefulness of ionic calcium measurements can as yet only be inferred from small groups of patients studied as research projects on research type instrumentation (12,18,19) and the pragmatic role of these measurements has yet to be established as the result of widespread clinical experience (a statement on medical usefulness).

Because a positive statement about 1) the molecular biology 2) the analytical chemistry and 3) the medical usefulness is possible for total calcium in serum it has already been possible to develop analytical goals and quality requirements for this analyte (14). However the lack of widespread experience with proven analytical methods to quantitate free calcium (15-17) is the major reason why goals for this even more important body

constituent cannot be developed at this time. In this example, analytical quantitation was adequate for research workers to gain the original molecular insight; however, the analytical methods for making meaningful daily measurements on patients is still to be resolved. Until this analytical problem of routine determinations is resolved it is impossible to provide the objective test data to answer pragmatically the question of its medical usefulness on any meaningful widespread basis.

THE NEED TO AVOID "FOSSILIZATION"

While agreements and standardization can lead to an acceptable degree of compatibility with time, they will also tend to focus excessive attention (including our laws and regulations) on what has in the past been acceptable. Since total clinical laboratory resources of human energy, skills and time are finite, we must continually ask if the accurate measurement of one particular analyte is still meaningful in light of the changing knowledge in biology, chemistry and medicine. For example, in the metabolism of bilirubin in the newborn (a molecular biology question) it now seems as if the reserve bilirubin binding capacity of serum and "loosely bound bilirubin" (analytical quantitation problems) may be the critical parameters to measure to avoid kernicterus (20) (medical usefulness questions). To the extent we direct too much attention and commit limited resources to efforts to standardize less meaningful parameters, the accuracy and compatibility we achieve could actually be counterproductive and retard the measurement of the newer, more biologically important analyte(s). Therefore, first priority efforts should be on those analytes for which each criterion points unambiguously to a universal patient need.

OPENNESS, COOPERATION AND COMMUNICATION

The key psychological elements upon which any national reference system must rest are openness, objectivity and effective communication. All interested parties either in the U.S. or elsewhere should have access to the concepts and facts upon which decisions are made. Such information must be actively communicated via publications which are readily available and widely circulated (e.g., in Clinical Chemistry). There must be real cooperation based upon the

appropriate blend of scientific competency, medical judgment and organizational abilities that each group can contribute. In particular, governmental representatives must be exceedingly cautious not to dominate policy boards and working groups but to cooperate as equals. If this is not the federal posture, these activities will rightly be viewed as regulatory and not as a cooperative voluntary standardization effort. It should be remembered that in the U.S. almost all effective and compatible measurement networks have come into being through the voluntary consensus standards process. Sufficient examples are available to assure that this process can also work for a clinical chemistry measurement network.

ORGANIZATION OF A NATIONAL REFERENCE SYSTEM FOR CLINICAL CHEMISTRY

The American Association for Clinical Chemistry supports and encourages the adoption of a coherent, national reference system for clinical chemistry measurements which will insure the biological relevance, chemical validity and medical usefulness of such data.

COMPONENTS AND INTERRELATIONSHIPS

As shown in Figure 1 this national system for clinical chemistry should be based upon:

1) The International System of Units (SI)

2) A hierarchal structure of interrelated

   a) methods (definitive, reference and working)

   b) materials (certified, matrixed, control, etc.)

3) Defined relationships between professional, industrial and governmental groups of scientific, medical and technologica persons.

SCIENTIFIC RESEARCH AND DEVELOPMENT

This system requires continuing research and development to establish the definitive methods, Certified Reference

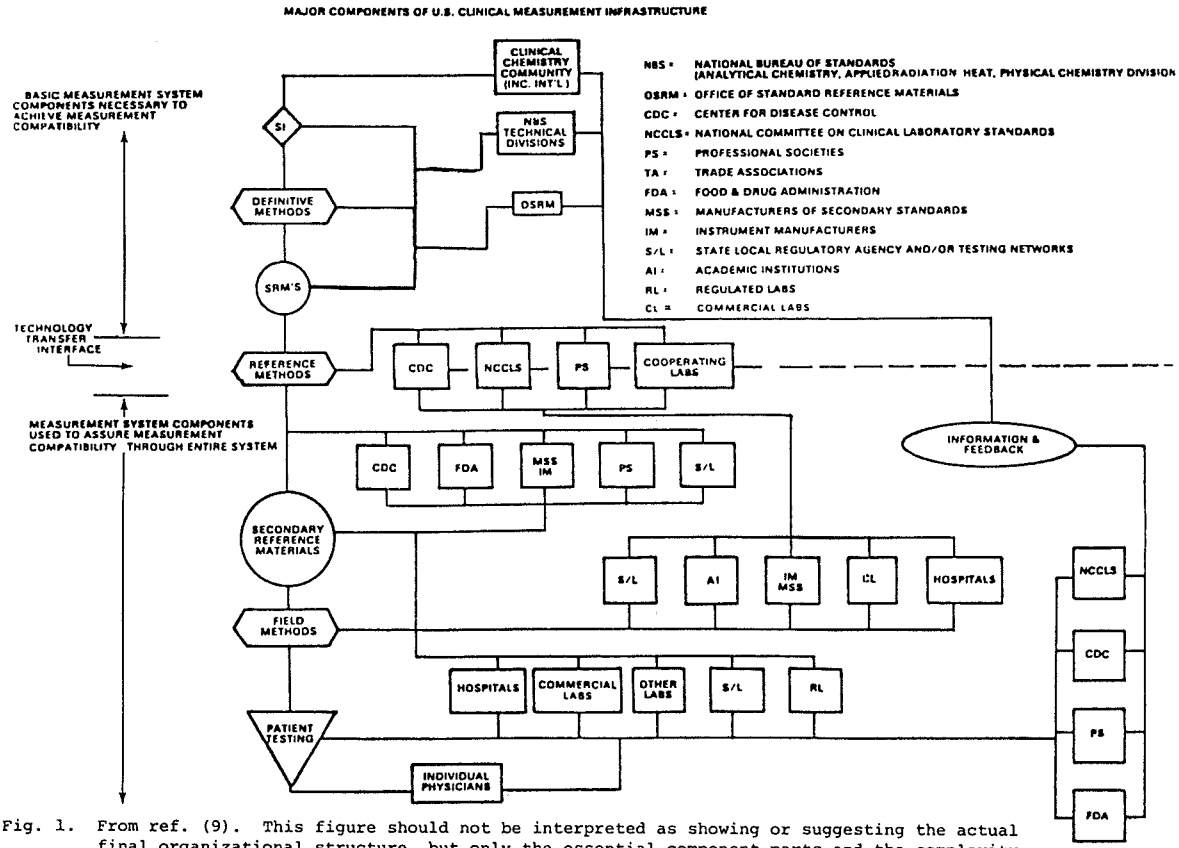

Fig. 1. From ref. (9). This figure should not be interpreted as showing or suggesting the actual final organizational structure, but only the essential component parts and the complexity of the numerous interrelationships.

Materials and reference methods for all major clinical chemistry constituents.

Existing federal laboratories at the National Bureau of Standards and the Center for Disease Control should expand their efforts to develop the scientific framework on which the reference system will be constructed. It is particularly important to actively seek industrial input from appropriate trade associations (HIMA, PMA, SAMA) and consensus groups (NCCLS) to insure that the introduction of improve accuracy standards for diagnostic products occurs smoothly and without economic dislocation.

ADVISORY PANELS NEEDED

Panels of experts from a variety of disciplines (clinical chemistry, pathology, medicine) should be assembled to provide priorities, realistic goals and review of programs and outputs of national laboratories concerned with the national reference system. Besides assuring that efforts of these national laboratories are directly supportive of the goals and functions of the national reference system such panels should also aid in making evaluations of the cost effectiveness and the benefits to the nation.

PRIORITIES

Priorities should reflect areas: (1) where improved accuracy, sensitivity and specificity could clearly affect patient well being (2) where improvement in accuracy may increase the liklihood of proper diagnosis (3) where proficiency surveys have shown a serious question of accuracy and precision to exist. Flexibility should be provided to allow for ready revision of priorities as new knowledge is achieved relative to the criteria of molecular biology (understanding of the underlying basis of the disease process), analytical chemistry and medical usefulness.

FUNDING

The impact of this national reference system for clinical chemistry measurements on numerous other health care activities requires assignment of a high priority to its further development. Funding for federal efforts should be

centralized in DHEW, which has the responsibility for overall federal budgeting in the health care field. However, with federal funding comes the chance of federal control, so that substantial input from the private sector should be sought, both from manufacturers and professional societies. AACC has contributed both financial support as well as volunteer professional expertise to these efforts in the past, and will most likely increase this commitment in the future.

In particular, funding (contracts or grants) is required to support: a) NBS progarams and outputs, b) CDC programs and outputs, and c) those clinical laboratories engaged specifically in studies of the validity and the transferability of reference methods and materials.

RECOMMENDATIONS

In respect to the analytical goals and organizational development of a national reference system, the American Association for Clinical Chemistry holds the following position.

(1) It supports and encourages the adoption of a coherent, national reference system for clinical chemistry measurements which will insure the biological relevance, chemical validity and medical usefulness of such data.

(2) The major goal and function of the system should be to achieve measurement compatibility within and among service laboratories in the U. S.

   a) The guiding philosophical principle should be the measurement of an analyte(s)' "true value" in each clinical chemistry service laboratory.

   b) The system should permit the traceability of the performance of routine clinical chemistry measurements to the definitive methods of undisputed accuracy.

(3) The system should be built upon elements which make it as universal as possible:

   a) the basic international SI units.

b) nomenclature for materials, methods and standards in agreement with WHO, IUPAC and IFCC (see Glossary).

c) cooperation and leadership at the international level in matters of reference materials and methods.

(4) The control and organizational structure of the national measurement system should re-enforce the cooperative and voluntary standards approach.

a) It must be essentially a neutral, scientific, voluntary organization and not a quasi governmental regulatory mechanism. NCCLS is an example of such a neutral organization and its Area Committee on Clinical Chemistry might be the basis for a controlling administrative organization.

b) Open decision making and active effective communication between all members and other interested parties should be assured by its operating needs.

(5) Professional societies such as AACC should provide major inputs to the system:

a) to help set priorities for the reference methods and Certified Reference Materials.

b) to identify the need for and to help select the specific reference methods to provide greater accuracy within clinical laboratories.

c) to provide administrative staff support to assure that the system remains a vigorous, voluntary consensus type organization serving predominately scientific/medical goals.

d) to assure feedback and active two-way communication through advisory panels, publications, conferences and solicited comments of member scientists.

(6) AACC supports the following federal agency relationships, providing advisory panels as described

are utilized to coordinate agency efforts with the national reference system.

a) National Bureau of Standards should:

1. be the central distribution source for all Certified Reference Materials including biological CRMs through its Office of Standard Reference Materials.

2. continue to be the lead government agency to develop definitive methods.

3. continue to develop high purity, well characterized Certified Reference Materials that are within the competency of its personnel.

b) Center for Disease Control should:

1. be the prime governmental agency responsible for reference methods.

2. continue to develop biological reference materials (e.g., enzymes) and other complex materials.

c) The Food and Drug Administration should utilize and support the scientific development of components of the national reference system, particularly definitive methods and Certified Reference Materials, by interagency agreements with NBS and CDC so that a scientific basis for regulating activity is established as distinct from the legal mandate.

MATERIALS COMPONENT

The AACC recognizes the need for the establishment of a hierarchy of materials which, through their interaction with methods, play a key role in any National Reference System. In consort with the IUPAC Commission on Physiochemical Measurements and Standards (21), AACC supports the use of the terms "Certified Reference Materials" and "Calibration and

Test Materials" to define materials of high purity (see Glossary).[1] These are two major subclasses of Reference Materials. Certified Reference Materials replaces the term "Standard Reference Materials" to define materials which are of high purity or contain definable substances and have been certified by a recognized body (e.g., National Bureau of Standards).

The prime roles of Certified Reference Materials are to: 1. help in the development of reference methods, 2. calibrate measurement systems, 3. help assure the long term adequacy and integrity of a quality control process. AACC believes that Certified Reference Materials should be developed and used in the field of Clinical Chemistry for each of these purposes. AACC also emphasizes the need for Certified Reference Materials in a biological matrix for clinical chemistry. Examples of available and commonly used calibration and test materials as well as further helpful discussion may be found in References 22-24.

CERTIFIED REFERENCE MATERIALS

AACC believes that Certified Reference Materials priorities should be set by clinicians and clinical laboratory personnel working in cooperation with NBS, through an expanded advisory committee system. Criteria for setting priorities include recognition of determinations: 1. where improved precision and accuracy clearly affect patient well being, 2. where improvement in accuracy may increase diagnostic sensitivity, 3. where proficiency surveys have shown a serious question of accuracy and precision to exist.

In general, Certified Reference Materials should be available for all analytes. AACC recognizes, of course, that it will be a task of many years to achieve this objective. Many Certified Reference Materials are, however, now available (22-25). These are principally compounds which can be prepared as high purity substances. Determination of

[1]The Commission also recognizes Primary Substances. These are fundamental substances for the realization of measurement scales; an example is found in the hyperfine transitions of cesium 133, used to define the second as a unit of time.

purity of these seems straightforward and goals of at least 99.9% appear to be achievable.

Other materials, however, such as NADH, cholesterol, bilirubin and hormones may be obtained from biological sources. While their structures are well known, assessment of purity is more difficult as is proof of stability. AACC believes that the fundamental chemistry of this type of compound should be thoroughly investigated prior to establishment of final purity figures. Under the IUPAC classification, there is no reason to believe that Certified Reference Materials (interim) could not be developed for such compounds.

There are many substances of indefinite or poorly understood composition, for example serum proteins, triglycerides, and enzymes. There is need for Certified Reference Materials for these substances as well. While molecular composition may not be specified, characterization on the basis of several selected criteria is feasible after reasonable purification. Materials of this type should be human in origin where practicable. If materials of non-human origin are used their properties should be carefully compared to those of human origin (5, 26). Matrix considerations may preclude the use of some human materials.

Finally, there seems to be a need for materials similar in intent to reference orchard leaves or liver powder. AACC believes, for example, that the feasibility of a reference serum representing an average normal human serum matrix should be investigated. Specifications for Certified Reference Materials should be set by NBS in cooperation with clinical laboratory personnel. Study of feasibility, need and cost should take place by these groups.

Biological Certified Reference Materials present unique difficulties since the chemistry of the process of purification may be poorly understood. Nevertheless, Interim Certified Reference Materials may be needed.

Validation of specifications should be an obligation of the NBS or another specifically designated laboratory. AACC recognizes that, ultimately, the need for Certified Reference Materials may outrun the capacity of NBS to produce and

maintain them. If multiple sources are required, rigorous quality assurance procedures defined by NBS should be established. A certificate from a source other than NBS should have identical credibility.

It should not be overlooked that there is a need for Certified Reference Materials to validate the accuracy of certain measurement devices. AACC believes that NBS should produce or continue to produce Certified Reference Materials for instruments such as analytical balances, spectrophotometers, fluorometers, thermometers and temperature measurement apparatus, pH meters, atomic absorption instruments, gas chromatographic devices, etc., at least on a selected basis (27,28).

BIOLOGICAL MATRIX MATERIALS

Biological matrix reference materials are intended for use as probes in the assessment of the reliability and performance of an analytical process. They shall be used to interrelate definitive, reference and routine methodologies and help define analytical biases associated with established methods. These materials will be used in evaluating the accuracy (freedom from bias) and the precision of candidate reference methods. They will be used to help understand and eliminate bias (matrix effects) in methods (both reference and routine) under developement or evolution. These matrix materials will also be used as sample-simulators in the transfer of reference method technology to routine assay techniques. Although these materials may be ideally suited for calibration and assessment of daily intra-laboratory quality control, this use is not the directed function of these materials; this application is clearly secondary to all the above functions.

An additional use for these materials will be the evaluation of the performance of diagnostic products and in selected interlaboratory testing. Their use in regular external quality control programs is not precluded but this is not a prime objective considered in defining their functions or specifications.

The desired composition of biological matrix reference materials will depend upon their intended use. The selection

of only a single matrix, even for the examination of one analyte, is undesirable. The nature of the information desired from the observed interaction between the material and the analytical technique should determine the characteristics of the matrix.

The matrix is defined as the entire milieu surrounding the analyte examined. Thus all other components, including other "analytes" not be estimated by the analytical procedure under investigation, shall be regarded as the matraix. The definition of the matrix is therefore different for each analyte under consideration.

The capability to define the matrix will vary among candidate materials. The definability is directly related to the simplicity of the reference material and therefore the most complete biological matrix will be the least definable. Candidates include:

a) Pure substances of known composition in a known matrix and solution (e.g., NBS SRM quality KCl in aqueous solution). Although completely definable, these standard solutions may serve only as bench marks upon which the magnitude of matrix effects may be quantitated.

b) Pure substances of known composition in a partially characterized (standardized) matrix and solution (e.g., NBS SRM quality KCl in highly purified, defined serum albumin in aqueous solution).

c) Partially characterized substances in a partially characterized (standardized) matrix and solution (e.g., candidate enzyme reference materials, or similar biologicals, in defined aqueous solution of serum albumin and water diluent).

d) Pure substances of known composition in a poorly characterized matrix (e.g., NBS SRM quality $CaCl_2$ in a full serum matrix).

e) Partially characterized substances in a poorly characterized matrix (e.g., candidate enzyme reference materials in a full serum matrix).

f) Poorly characterized substances in a poorly characterized matrix (e.g., serum or urine).

In all cases, despite the variable origin of component materials, the composition of the material should be known as completely as possible. Sources and nature of components and any modifications should be specified. Information on additives, antibiotics, preservatives, etc., must be stated even if the effects on analyses are unknown or presumed to be negligible.

As stated above the nature of the desired matrix is a variable dependent upon both the analyte and the analytical process. The characteristics of the candidate materials should be selected to optimally examine possible biases within the analysis system. Freedom from all matrix effects is a desirable but often unattainable analytical goal. An understanding and definition of specific matrix effects is a feasible alternative to the ideal situation. The least defined of biological reference materials will expose the sensitivity of an analytical process to matrix effects and materials of greater definition will be used to isolate and define these effects. It must be remembered that, with biological materials, matrix effects are both complex and, in general, common. Matrix effects may include:

a) Interfering chemical effects. Materials present in the biologic matrix may result in interferences due to the nonspecificity of given analytical technique (e.g., the interference of galactose the o-toluidine technique for estimation of serum glucose, or the presence of glutamate dehydrogenase influencing the results in the measurement of aspartate aminotransferase activity with the NADH/malate dehydrogenase coupled system).

b) Direct chemical effect upon the analyte. A component of the matrix affects the measurement by directly influencing the activity of the measured analyte (e.g., the effects of increased serum Mg^{2+} or Zn^{2+} upon serum alkaline phosphate activity, or the presence of phospholipids in the estimation of glutamate dehydrogenase activity.

c) Physical interaction. Analytes may be bound to, or interact with, serum proteins or other serum components, influencing their analysis (e.g., the effects of thyroid binding globulin or albumin on the protein binding assay for thyroxine or the effects of phosphate on the atomic absorption technique for measurement of calcium concentration).

d) Artifactual instrument related effect. Component(s) in the matrix produce a response indistinguishable from the analyte in the measurement (not chemical) process (e.g., bilirubin or lipemia in certain methods not employing sample blank correction).

e) Viscosity effects. The total solid of the matrix sample results in altered flow rates, atomization, or aspiration (e.g., the effect of protein on the aspiration rate of fluids into flame emission photometers).

f) Donnan and other charge related effects. Electrostatic forces between analyte(s) and charged, high-molecular-mass species may result in exaggerated or diminished mobility of the smaller molecular mass analyte(s) (e.g., the Donnan membrane effects observed in automated techniques employing dialysis for estimating serum iron).

The selection of the appropriate biological reference materials is dependent upon the nature of the analyte, analytical procedure, calibrator, and the specimen to be analyzed. All of these components must be interrelated; a change in the choice of calibrator may necessitate a change in the choice of biological matrix reference material.

The utility of serum simulators will depend upon the ability of the matrix (in this example, serum albumin) to emulate or mimic the effects due to the complete serum matrix. Consideration should be given to other possible biological (alternate serum fractions) or nonbiological (polyvinylpyrrolidone or polyethyleneglycol) matrices. Consideration should be given to the manipulation of a full serum matrix (through dialysis, membrane filtration, or ion exchange chromatography) resulting in the preparation of analyte-free material which retains essentially a full

component of large molecular weight species.

Consideration should be given to nonhuman sources for biological matrices. The lack of high concentrations of β-lipoprotein in bovine serum may make this material the preferred base for lyophilized samples. The use of bovine, or other nonhuman source, would also reduce the chances of contamination with hepatitis associated antigen.

AACC feels that the achievement of completely satisfactory reference methods will be difficult or impossible until matrix effects are better understood. Working with the above classification, AACC believes that funding should be available at NBS and CDC for research support of matrix effects. Both internal and external support should be available.

RECOMMENDATIONS

The AACC:

1) Believes that a national reference system for clinical chemical measurement should be established.

2) Supports IUPAC nomenclature and recommends the use of the terms Primary Substances, Certified Reference Materials and Calibration and Test Materials. The general term for all is Reference Materials. AACC also recognizes the important role of Reference Materials in Biological Matrices. AACC is dedicated to help in the establishment of these materials.

3) Supports the primary role of NBS in providing Certified Reference Materials as well as the development of definitive methods. AACC understands that this will require extensive research, and NBS is actively engaged in such work.

4) Believes that the Center for Disease Control should continue to be responsible for biological reference materials and other complex materials not provided via NBS.

5) Believes that priorities should be set by appropriate advisory panels working closely with NBS and CDC. Consensus on priorities should be based upon scientific documentation.

6) Believes that continued development of Certified Reference Materials for analytical instruments used in the clinical laboratory is needed.

7) Believes that understanding of matrix effects as contributors to bias is necessary and research support should be available. Analytes in a biological matrix should be made generally available as Certified Reference Materials.

METHODOLOGICAL COMPONENT

Clinical chemistry encompasses a range of analytical procedures which are required for the qualitative and quantitative measurement of clinically significant components of physiological fluids. These analytical procedures are a result of the application of diverse analytical principles to best solve an analytical problem in the clinical chemistry laboratory. These analytical principles include photometry by absorption, fluorescence, reflectance, flame emission, flame absorption and chemiluminescence, the vast modes of chromatography separation, mass spectrometry, radiochemistry, electrochemistry, titration, centrifugation (preparatory and analytical) and gravimetric measurements. The application of the above principles of analysis has resulted in a diversification and proliferation of methods either within or across the various categories of analytical principles. This proliferation of methods has been dictated in part by the need for better specificity, accuracy, reliability, ease of operation, economy, efficiency or simply the exercise of scientific curiosity. The goals for the development of a new method such as better efficiency or economy are often exclusive or partially exclusive of other goals such as specificity and reliability. Thus it is necessary that reference methods be established to serve as a measure of the acceptability of new methods.

The fundamental description of any method must include a complete statement of the chemical principles on which the

method rests, the exact mathematical relationships between results and measurement parameters and the relationship between these and fundamental units in the Systeme Internationale.

The working description of any method consists of detailed step-by-step instructions for making the reagents, setting up the necessary equipment, calibrating and operating the necessary instruments, preparing the sample, performing the chemical steps of the procedure, calculating the results, estimating their uncertainty and expressing the results in proper form.

DEFINITIVE METHODS

Definition and description

A "Definitive Method" is an analytical chemical procedure, for the measurement of a specified analyte in a specified material, which is known to give essentially the "true value". The procedure and each of its steps have been tested thoroughly and shown to be free from systematic error with a high degree of reliability. The procedure has been documented meticulously. In short, it is the best known available yet technically feasible method for the determination in question. Its practicality for routine use is not a consideration.

Utilization

For analytes of clinical importance definitive methods are necessary to provide authoritative answers to questions involved in the development of reference materials and reference methods. They have the same relationship to reference methods and routine working methods that Certified Reference Materials have to reference materials and routine calibrators, respectively. The same relationships also hold with respect to difficulty and frequency of use. The definitive method may require large amounts of time and resources to develop, verify and document, as does the Certified Reference Material and, like it, must be used selectively and sparingly for economic reasons.

Application

Definitive methods will be performed for carefully selected analytes on well defined materials which will form reference materials for broader application. The key characteristic of definitive methods, namely their degree of error, must be known, and materials for analysis must be chosen, prepared, handled and stored so as not to increase that error. Thus, to a significant degree, the material analyzed must be carefully specified in the working description of any definitive method. Definitive methods are so expensive to perform that it would be wasteful to apply one to anything other than a sample from an amount of material large enough so that all interested parties may obtain aliquots. This sample must be representative of the whole of the material to a degree of certainty which exceeds that of the definitive method. Similarly the homogeneity of the whole of the material must be uncertain to a negligible degree, as must its stability. The packaging for further use of portions of this material must not introduce significant uncertainty. All of these features must be assured by a complete working description which states how to achieve them reliably.

Production and use

Developing and using definitive methods are both specialized feats which require the ultimate in technical expertise. Sites capable of such performance are limited. The National Bureau of Standards, the World Health Organization and a few like bodies are the only ones dedicated to developing and using such methods as primary tasks. While it is important that mechanisms exist which can exploit the expertise of other bodies, industrial, academic or governmental, that can and might develop and use definitive methods, it would be unrealistic to count on them to fill any great fraction of the need or to attack the need in any systematic way. Thus these dedicated government bureaus must be given the mandate and support for development and use of definitive methods.

REFERENCE METHODS

Need

Reference methods are needed for clinical chemistry laboratories, government agencies and manufacturers in order to have a recognized base for comparison and to serve as a measure of the acceptability of new methods. The CAP has designated "comparative methods" to judge survey results (29). For the laboratory, a reference method means a procedure which is either in routine use or which a could, if it wished, put into operation to validate a routine method. In major centers or during important studies, such as population surveys, it would be desirable to use the reference method directly. If this is not practical, a method giving values as closely related to the reference method as possible should be used. For manufacturers of automated instruments and analytical kits the reference method could be the means by which the performance of their products could be established. Values cited on secondary reference preparations (calibrators and controls) should be related to reference materials through reference methods.

Requirements

Features requisite in a reference method are: (1) It should be based on sound chemical principles which are well understood in relation to the analyte. (2) The method should have minimal bias relative to the corresponding definitive method. It may be that the reference method and the definitive method are the same, if the definitive method is sufficiently simple (see (5) below). (3) The bias, if any, should be known and should be constant with specimens of different characteristics. (4) High precision should be achievable, and interferences should be minimal and as well documented as possible. (5) The method should be simple and economical enough in terms of instrumentation, reagents and personnel time that it can reasonably be established in working laboratories, certainly at least in larger centers, as well as in the laboratories of small manufacturing concerns and government agencies.

It is apparent from the dynamic spectrum of existing methods that defined and verified reference methods do not

exist for all major classes of clinical chemistry procedures. The establishment of a reference method requires a definitive method and a Certified Reference Material. For some complex substances, such as enzymes or the total protein of serum, availability of definitive methods and Certified Reference Materials in the strict sense is remote in time and may never be achieved. Therfore, AACC recommends the definition of three classes of reference methods, in addition to definitive methods, as those which (A) have been verified, (B) are awaiting verification and (C) will probably never be verified (see 5.0, Glossary).

Steps to accomplish a reference method

The eight general steps for arriving at a reference method outlined in a preliminary CDC paper appear logical. These are (1) selection of analyte, (2) definition of analyte, (3) specification of performance requirements, (4) selection of a candidate method, (5) development and validation, (6) transfer, (7) acceptance, (8) propagation and maintenance. Comments on these steps are in order.

Step 1. Priorities must be flexible, since goals may change and resources are limited. For instance, is bilirubin concentration or reserve bilirubin capacity the significant analyte in neonatal blood serum? Costs and effectiveness in improving patient care are important considerations.

Step 2. Even the definiton of the analyte may be subject to change, for instance when it is recognized that a hormone is excreted as both free and conjugated forms rather than as the free form alone.

Step 3. Specification of requirements can only be recommended, and must incorporate some flexibility in the interests of reality. In addition to desired accuracy and precision, the concentration ranges of particular significance should be cited. Medical usefulness estimates may be coupled with intra- and interindividual variability and with results of surveys of laboratory performance to give a starting point for analytical specifications, but the guiding concern must be good analytical principles and procedure. Accuracy should be the prime consideration. To base analytical specifications entirely on estimates of

current usefulness would discourage progress in scientific understanding of disease.

Step 4. A candidate method may be selected directly from a published and accepted method, such as flame photometry for potassium, or may be developed by modification of existing methodology to adapt it to the requirements of clinical analysis. Examples are given below. In developmental work efforts should be made to reduce bias relative to the definitive method and to reduce matrix effects and interferences observed with clinical specimens. If these effects cannot be eliminated, they should be studied to determine their causes and in so doing be made predictable and reasonably constant. Publication of findings at frequent intervals is essential.

Step 5. Development, if needed, should be performed in a single laboratory with only occasional corroboration by a colleague. Validation, on the other hand, should be accomplished by round robin analyses among a number of laboratories, eight to twelve, which have experienced leadership. A coordinator should lead the development of detailed protocols, which should be thoroughly pretested by round robin mailings to assure clarity of directions. The cooperating laboratories then perform the protocol exactly as prescribed, without innovation, so that the results may be statistically treated. The statistical treatment must be carefully planned. Again, publication of findings should be frequent in order to expose the behavior of the method to public scrutiny.

Steps 6, 7, 8. Transferability, acceptance and maintenance are intertwined. Comments on performance of the procedure in potential user laboratories should be fed back to the coordinator of step (5); major results should be published. Acceptance entails a consensus agreement. The definition of consensus as listed in the Glossary is recommended, in which all conflicting arguments have been considered and resolved. Promulgation through an inter-group association such as NCCLS is a promising means to achieve consensus.

Propagation will be accomplished through such open publication plus the consensus mechanism which entails publication as a formal document. Maintenance of the method

should be through the revision process built into the consensus "standard"; a chain of interest should be maintained through the continuing involvement of the scientist coordinator or team leader of the development and validation steps.

EXAMPLES OF DEVELOPMENT OF REFERENCE METHODS

Two examples are given, the first, potassium, to illustrate a well-defined analyte with accepted methods in use and the second, total protein, a complex analyte with numerous versions of a basic method in use. Efforts are underway with more complex and unstable analytes such as aspartate aminotransferase but these are not reviewed here in the interests of brevity.

(1) Potassium (a potential Class A Reference Method, currently Class B)

Potassium ion in serum and other body fluids rates a high priority since this is a common test on which many medical decisions are made. Definition of the analyte in terms of current understanding is simple -- total potassium -- since only ionizable potassium is found in fluids and there appears to be no heterogeneity or lack of exchangeability of the potassium in different compounds. The performance goal of 2.8% CV proposed by the 1976 Aspen Conference (30) is below the current performance estimates from surveys of 4-4.5% CV. A well accepted version of a procedure using flame photometry methodological principles is at hand (31), however, and only improved technology of application is needed.

(2) Total protein in serum (a Class C Reference Method, since a definition of the analyte appears unlikely)

Total protein of serum is a complex analyte, containing at least 50 distinct molecular species ranging in composition from 2% to 100% peptide, the remainder being carbohydrate or, in a few cases, lipids. In 1963, over half of the results for total protein in interlaboratory surveys were found to be

unacceptable (32), and results of later surveys of 3-7% CV were well above the recommended and achievable analytical goals of 1.5-2% CV (30). Hence a moderately high priority was justified.

The AACC Standards Committee in 1968 (33) proposed that total protein analyses be based on the peptide component of complex proteins, that the biuret procedure be employed as a measure of total peptide and that a pure bovine serum albumin solution be used as a standard material. These proposals have in essence been followed and reference method (Class C) is nearing fruition; the story of its development illustrates both intergroup cooperation and entrapment in several pitfalls.

The specifications for a bovine albumin solution as a surrogate standard for total protein were published as an NCCLS Approved Standard in 1973 (34). Upon recommendations of its Advisory Committee, NBS let a contract for such material and has certified it as SRM No. 927 (a CRM). No loosening of the specifications was required for commercial production, although production was halted for nearly a year by the finding of unexpected amounts of free amino acids in the purified albumin preparation. Conferences and tests by protein chemists resolved the origin of these amino acids as proteolysis by leukocytic enzymes. An additional heating step inactivated the enzymes; improved collection procedures of bovine serum could eliminate them. Thus, a Certified Reference Material, albeit a surrogate one, is available for total protein.

A second problem was that the recommended biuret method proved nonlinear at the protein concentration of the Certified Reference Material (70 g/liter). Members of the AACC Standards Committee recommended a stronger reagent, which was tested in a round robin and the results published (35). CDC assumed coordination of the next stage of round robins, and Drs. Bayse and Carter of CDC together with AACC Standards Committee chairman Doumas are first assuring that the reagents and reaction conditions are optimal. The protocol for this interlaboratory validation is highly detailed and includes spectrophotometry standards which had proved their value in the first round of tests. The revised biuret procedure will be circulated for consensus approval by

means of a revision of the NCCLS ASC-1.

PROVISIONAL REFERENCE PRINCIPLES

Scope and Objectives

The length of time and amount of resources required to establish reference methods makes it uncertain that reference materials or reference methods, even those of Class C, will be developed in the near future for such analytes as enzymes, therapeutic drugs, steroids and hormones. Provisional methodological principles can provide the needed first line of standardization. In their selection, however, the approach must use "Good Science" rather than arbitrary or "arm chair science".

Establishment of Provisional Methodological Principles

The delineation of guidelines for the establishment of candidate methodological principles should include the following steps:

1) Define the analytes for which reference methods do not exist, and the methods for which standard reference materials do not exist.

2) Define the possible existing materials and methods which may be candidates for provisional reference materials and methodological principles.

3) Assemble a reference list of papers and committee actions directed towards the establishment of accuracy goals for the acceptable performance of methods and materials. The efforts of the NBS, NCCLS and Westgard et al. (36, 37) should be considered.

4) Attempt to define or establish a useful accuracy goal for methodological principles.

5) Delineate the criteria for provisional reference materials.

6) Consider the possible objective, the areas of influence and the potential positive input of the other

participating groups as a possible first step to establishment of consensus mechanism.

7) The net result of the above efforts should be a written guideline for methodological principles and should include the following features:

   a) The method which is chosen to illustrate the principle must be written.

   b) The method must be in common use by sufficient number of clinical chemistry laboratories to determine performance criteria.

   c) The method must be based on good analytical or bioanalytical chemistry techniques. It should be recognized that several methods may qualify as provisional reference methodological principles by satisfaction of the established guidelines.

   d) The limitation of the method such as interferences must be established for commonly encountered matrix components such as bilirubin, hemoglobin, lipids, and common analyte analog compounds. The bias must be established. An idea of likely bias should be available.

   e) Limitations of the method due to the measurement system must be defined: e.g., detector sensitivity, flame temperatures, fuel mixtures, band pass, stray light, flow rates, etc.

   f) The precision (inter- and intra-laboratory) should be established.

   g) Linearity of the method, substrate to reagent ratios, etc., must be established.

   h) Specimen stability and handling with regard to the analyte must be established.

   i) Accuracy and precision should be within established guidelines as delimited on the lower side by rigorous propagation of error analysis of total system random

error and consideration of total systemic error and on the upper side by consideration of clinical usefulness of the data (9, 38).

LIMITATIONS ON THE USE OF PROVISIONAL REFERENCE PRINCIPLES

a) Provisional methodological principles may be useful, if interorganizational approval is achieved, as reference positions for measuring performance of clinical instruments, reagents or test kits by manufacturers, or for specifying principal assigned values for calibrators and control materials.

b) The provisional nature of the selections must be continually recognized, and their use must be only a temporary or interim measure pending the development of validated reference methods and materials. In particular, incorporation of provisional methodological principles into regulatory product class standards for individual analytes is discouraged, since the very rigidity of these standards would retard possible progress through modification or substitution of improved procedures.

c) Provisional methodological principles are not intended as a committed step toward the development of a reference method or material although this possibility is not excluded. In the development of reference methods or materials no grandfather clauses should be permitted and existing methods qualified as provisional methods and materials should be reevaluated to promote a provisional method to a reference method. In many cases this would not require major reevaluation of the field method.

ESTABLISHMENT OF A REVIEW MECHANISM

The impact of the provisional methodological principles can best be judged by proficiency examination. Proficiency testing and questionnaires designed along the lines of continuing education as currently conducted by CDC and CAP are useful vehicles to measure the effectiveness and make recommendations for improvement of the provisional reference program. The above goals are educational and not regulatory in nature.

RECOMMENDATIONS

The AACC:

1) Supports and encourages the adoption of a coherent, national reference system for clinical chemistry measurements which will insure the biological relevance, chemical validity and medical usefulness of such data.

2) Supports the concept that laboratory methods be ultimately based on definitive methods.

3) Supports NBS as the lead agency to develop definitive methods.

4) Urges that the FDA continue and increase its program of support for development of definitive methods.

5) Supports the need for reference methods as intermediaries between definitive methods and the operating laboratory.

6) Supports CDC as the prime governmental agency responsible for reference methods.

7) Favors the use of sound criteria rather than consensus agreement or balloting for the selection of reference methods and of provisional methodological principles.

8) Urges that provisional methodological principles be considered a temporary expedient for interim use only, and that such selections not be given undeserved credence by incorporation into product class standards for individual analytes or be made the obligate basis of selection of reference methods.

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5.0 GLOSSARY

The definitions listed below follow international usage where possible, but some definitions have been modified and some new terms have been introduced to accommodate the purposes of this Paper. The term "standard" has been avoided owing to the ambiguity inherent in its several uses as a document, as a criterion of performance, and as a comparison solution.

Sources

(1) From IFCC Provisional Recommendation, Stage 2, Draft 2, December 1974.

(2) Modified from (1).

(3) From IFCC, Expert Panel on Theory of Reference Values, IFCC Newsletter, February 1977.

(4) Modified from (3).

(5) Radin, N (1967) What is a Standard? Clin. Chem. 13:55.

Accuracy. Agreement between the best estimate of a quantity and its true value. It is measured in terms of error. (2)

Aliquot. A measured portion of a sample, having the composition and properties of the sample from which it is taken. (A general term that applies to proportions of solutions or homogeneous mixtures.) (2)

Analyte. The component to be measured. (1)

Analytical method. See method, analytical.

Analytical range. See range, analytical.

Bias. Same as systematic error. (2)

Calibration. Execution of a procedure or protocol which characterizes the mathematical relationship between the known analyte concentration in a "calibrator" and the analyzer response for the time interval between calibrations.

Calibration and test material. See material, calibration and test.

Calibrator. Material or solution with which the sample is compared in order to determine the concentration or other quantity. (2)

Carryover. The systematic error produced by a given sample in a series upon adjacent samples. (2)

Certified reference material. See material, certified reference.

Certified value. See value, certified.

Class, reference. A group of individuals in a described state of health or disease. A class may be composed of one individual either to act as a reference for himself or for another individual. (4)

Consensus. Compromise agreement which is acceptable to all participants. In reaching this agreement all participants should have been furnished all available data, and all comments and minority reports resolved or incorporated. Votes taken, if any, should be as straw polls only. (Proposed by AACC.)

Control material. Material used for quality control purposes. (1)

Control specimen, control solution. Specimen or solution which is analyzed solely for quality control purposes, not for calibration. (1)

Definitive method. See method, definitive.

Definitive value. See value, definitive.

Detection limit. The smallest single result which, with a stated probability (commonly 95%), can be distinguished from a suitable blank. The limit may be a concentration or an amount and defines the point at which the analysis becomes just feasible. (1)

Distribution, reference. The mathematical function deemed suitable to represent the probability distribution of the observable values in a reference class. This distribution is estimated from the values of the sample group. (3)

Error. The discrepancy between the result of the measurement and the value of the quantity measured. (Notes: The value of the quantity measured is a comparison value equal, according to the particular case, to the definitive method value, the reference method value, or the average or results of measurements by other specified methods.)

External quality control. Procedure of utilizing, for quality control purposes, the results of several laboratories which analyze the same specimen(s). (1)

Group, sample. An adequate number of individuals selected to represent the reference class. (3)

Imprecision. Standard deviation or coefficient of variation of the results in a set of replicate measurements. The mean value and number of replicates must be stated, and the design used must be described in such a way that other workers can repeat it. This is particularly important whenever a specific term is used to denote a particular type of imprecision, such as between-laboratory, within-day, or between-day. (1)

Interference. The effect of a compound(s) other than the analyte on the measurement of the analyte. (2)

Internal quality control. Procedure of utilizing the results of only one laboratory for quality control purposes. (1)

Interval, reference. An interval selected for interpretive purposes in the reference distribution. (3)

Material, calibration and test (CTM). See material, reference. (IUPAC)

Material, certified reference (CRM). A reference material accompanied by, or traceable to, a certificate stating the property value(s) concerned, issued by an organization which is generally accepted as technically competent (similar to current NBS Standard Reference Material (SRM). (ISO)

Material, reference (RM). A material or substance one or more properties of which are sufficiently well established to be used for the calibration of an apparatus or for the verification of a measurement method. Generally, any reasonably small part of a reference material sample should exhibit the property value(s) established for the reference material as a whole, within the stated uncertainty limits. (ISO)

Method, analytical. Set of written instructions which describe the procedure, materials, and equipment, which are necessary for the analyst to obtain a result. (1)

Method, definitive. An analytical method that is capable of providing highest accuracy among all methods for determining that analyte, and of sufficient accuracy for the stated end-purposes of the definitive method.

Method, reference. An analytical method whose accuracy and precision are sufficient, as demonstrated by direct comparison with the definitive method, and whose low incidence of susceptibility to known interferences is thoroughly documented so that the stated end-purposes of the reference method may be achieved. (Proposed by NBS.) A method validated against a definitive method would be further termed a Class A reference method. (Proposed by AACC.)

Method, reference (Class B). A method which is believed to be of the caliber of a reference method except that it has not yet completed the process of evaluation with a

definitive method and a CRM. (It is similar to the term interim reference method.) (Proposed by AACC.)

Method, reference (Class C). A method which is believed to be of the caliber of a reference method except that no definitive method and materials are likely to become available. (Proposed by AACC).

Precision. Agreement between replicate measurements. See imprecision. (2)

Pure. Free of other substances, to a degree consistent with cost and intended use, as judged by tests of known sensitivity. (5)

Quality control in clinical chemistry. The study of those errors which are the responsibility of the laboratory and the procedures used to recognize and minimize them. This study includes all errors arising within the laboratory between the receipt of the specimen and the dispatch of the report. On some occasions, the responsibility of the laboratory may extend to the collection of the specimen from the patient, and the provision of a suitable container. See external and internal quality control. (1)

Random. Due to unattributed causes; not affected by any kind of systematic selection or arrangement. (1)

Range, analytical. The range of concentration or other quantity in the specimen over which the method is applicable without modification.

Range, reference. All the observable values of a particular quantity in a reference class.

Reading. The value indicated on the scale of an instrument or analytical device.

Reference class. See class, reference.

Reference distribution. See distribution, reference.

Reference interval. See interval, reference.

Reference material. See material, reference.

Reference method. See method, reference.

Reference method value. See value, reference method.

Result. Final value obtained for a measured quantity after performing a measuring procedure including all sub-procedures and laboratory evaluations. (1)

Run. This usually refers to a set of consecutive assays performed without interruption. The results are usually calculated from the same set of calibration material readings. However, this definition may not be universally applicable, and in these cases the word series should be used after defining it. (1)

Sample. The appropriately representative part of a specimen which is used in the analysis. This sample should be called a test sample when it is necessary to avoid confusion with the statistical term "random sample from a population". (1)

Sample group. See group, sample.

Sensitivity. The ability of an analytical method to detect small quantities of the measured component. It has no numerical value. See detection limit. (1)

Specificity. The ability of an analytical method to determine solely the component(s) it purports to measure. It has no numerical value. It is assessed on the evidence available on the components which contribute to the result and on the extent to which they do. (1)

Specimen. Material available for analysis.

Systematic error. An error which, in the course of a number of measurements of a given value of a quantity made under constant conditions, remains constant, and which, when conditions are changed, varies in a predictable manner. (Proposed by AACC.)

Value (of concentration or other types of quantity in calibration standards, control specimens, etc., used in the following terms). The method by which the value is obtained must always be stated. (1)

Value, certified. Value certified by some official body subject to conditions established by that body. (1)

Value, definitive. A value given by a definitive method. It is the best available estimate of the true value. (2)

Value, reference method. The most probable value derived from a set of results obtained by the reference method. (2)

Value, true. Term considered to have a self-evident meaning requiring no definition. In practice, true values are approximated by definitive method values. (2)

AMERICAN SOCIETY FOR CLINICAL PATHOLOGISTS

POSITION PAPER

The American Society of Clinical Pathologists (ASCP) welcomes the opportunity to participate in the Conference on a National Understanding for the Development of Reference Materials and Methods for Clinical Chemistry. The ASCP position proposes that the role of the Conference is to develop rational criteria for the discovery of medical needs and relevance and to define various levels of materials and methods necessary to meet those medical requirements. Representatives from ASCP participating in the seven working groups have in addition submitted tentative or draft positions discussing the technical elements as pertains to their respective working groups.

This National Conference has been organized for the purpose of improving the performance of clinical chemistry laboratories. Implicit in the title which includes the word clinical is the intimate relationship with health care of human beings. In reality the purpose of the Conference is to assist clinical practitioners in both diagnosis and therapy through improvement of clinical chemistry laboratory performance. The Conference should always remember that its primary role is directed toward problems of health care and not necessarily chemistry for chemistry's sake.

With the rapid growth in both the variety and number of tests performed in clinical chemistry laboratories, there has developed an increasing awareness and concern over the quality of these tests. While surveys and proficiency testing schemes give insight into the question of accuracy and precision, there have been no standardized reference yardsticks gainst which a laboratory could gauge and evalute a given method.

During the last several years, a few defined standard materials have been developed and made available to clinical laboratories by the National Bureau of Standards. Individuals and professional organizations have proposed terminology to define levels or grades of reference methodology and materials. Government has been assigned the responsibility to regulate the quality and claims of laboratory apparatus, materials, and reagents.

All of these activities, whether collective or by individuals, seem to cry out for references against which

apparatus, materials, reagents and production-line methodologies can be judged. These standards, unfortunately, when available, will have little effect on assuring that such apparatus, materials, reagents, and methods are being properly performed or utilized. Only proficiency surveys can test such a question. The reference guides in materials and methods will only state what is possible when the tools available in the clinical laboratory are used properly.

At the current state of the art in clinical chemistry not every analysis can or need be judged against absolute reference materials and methods. Therefore, one needs various levels of absoluteness which will be established to judge operating methods and materials. Today by peer consensus such differences in degree exist. The analysis for serum calcium has both a material (the standard) and a method that are so well defined, so understood, and free of interference as to be classified at the highest level of absoluteness. On the other hand, serum immunoglobulins are routinely determined using standards and methods which are ill-defined. The standard for immunoglobulins has been established by fiat rather than by being traced to a fundamental measurement such as weight.

Functioning in a biological world, clinical laboratories know from experience that some measurable parameters need high levels of analytical performance while others need only qualitative yes or no answers. Therefore, clinical chemistry laboratories need several levels of reference material and/or methods against which they can evaluate their on-line analytical systems. For the future to assure practical clinical chemistry laboratory operations, the reference judging systems need flexibility based on rational scientific principles.

This National Conference should make a concentrated effort to establish criteria which define various types of standard materials, and levels of methodology. These definitions should stand by themselves. Each type or level of material and method would have assigned ranges for accuracy, precision, stability, influence by contaminants, types of interferences, etc.

Assuming that such criteria for various levels were in

existence, how would a given laboratory select which material and/or method against which to test apparatus, reagents, and/or proposed methods? Does the parameter or analyte under investigation need to be judged by an almost-perfect system of material and methods, or would a qualitative yes or no suffice? Where can a laboratory turn to answer that question? What criterion rules exist to evaluate the degree of performance needed to medically utilize the parameter? At the moment there is no system that can give such answers.

Again, the Conference has another critical and invaluable role. Because a large peer group who have collectively contributed immensely to the advancement, improvement, and understanding in clinical chemistry is gathered together, there would seem to be a unique opportunity to answer the above questions. The Conference participants need to establish a system and the criteria which would define the accuracy and precision for a given analyte. The levels set for precision and accuracy should be based on what is medically useful and necessary in order to properly diagnose and treat pathological conditions. In essence the established levels should be relevant to medical demands and not only to what is analytically possible. It would seem rational that not only input from the clinical chemistry arts is necessary but the Conference should consider soliciting the thoughts of clinical practitioners such as internists, surgeons, general practitioners, etc. Such a discovery system or approach would immensely assist the clinical laboratorians in deciding how much time, effort, and money must be spent to obtain an appropriate result which would be medically useful. This is not to say that the Conference should on its own discover or establish what is needed, but should create a system and the ground rules by which such needs would be established.

With the establishment of the above system we can discover what is needed in terms of accuracy and precision for a given parameter or analyte. The clinical laboratory, industry and manufacturers and regulatory agencies can then select an appropriately defined material and method which would be utilized to test on-line systems. Operationally, there are difficulties. For example, the discovery process may recommend that for an analyte a particular level of accuracy and precision is required in order to make the

results of the analysis medically relevant. That recommendation would establish the level or type of reference materials and methods which would be capable of judging production methods as being adequate to meet the medical recommendation.

Initially, there may not exist such a material or method. Then an ad hoc group or individuals, if so inclined because of the medical need, would engage in appropriate investigation and development to establish such a method. Assuming the Conference is successful in adopting appropriate criteria for various levels of materials and methods, then the result of the above investigations will be judged against a set of rules previously accepted by peers in clinical chemistry. Without those rules, any proposed reference for a standard or method used to evaluate routine laboratory methodologies for an analyte will face continued acrimony.

The Conference has two primary roles: establishing appropriate criteria for various levels of standard materials and methods and establishing a system which would ascertain what is medically relevant and necessary with respect to the clinical chemistry laboratory.

Another role of equal importance is the establishment of a system for communication between the practitioners of clinical chemistry, medical practitioners, manufacturers, and regulatory agencies. This system of communication or organization would suggest the establishment of appropriate ad hoc committees to assist in the discovery process and for reviewing proposed reference methods to see if they fit within the criteria for the various levels of absoluteness with respect to materials and methods. The communication system would include the organizational participants at this Conference, each contributing its expertise. The working group directed toward organization (communication systems) may seriously consider inviting other professional or technical organizations to participate. The communication aspects of this National Conference are more nebulous than those of groups discussing analytical goals (the discovery process) and materials and methods. Mechanisms for creating ad hoc groups for investigation and development, the establishment of referees to resolve discrepancies, and for adoption and approval, all are difficult questions that may

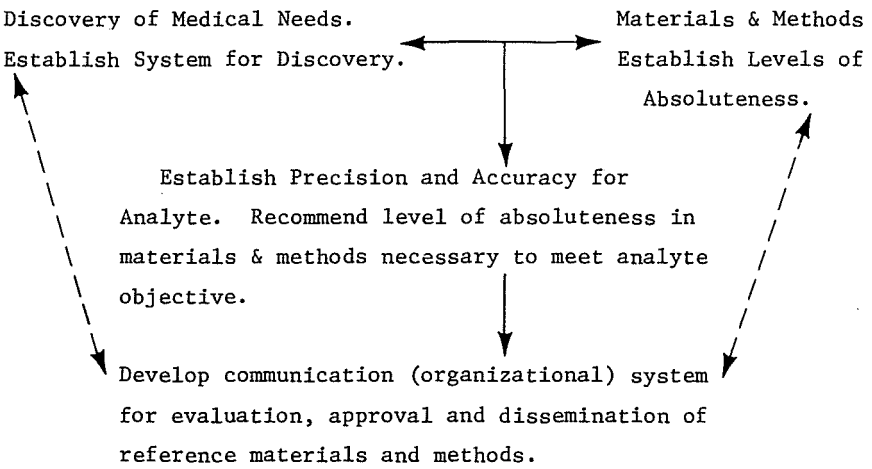
impinge the territorial prerogatives of Conference participants.

In the opinion of the ASCP, this Conference has the unique opportunity to establish ground rules which will be accepted by the large peer group. These ground rules should immensely assist all those concerned with health care, and in particular with clinical chemistry laboratory performance in techniques which will permit them to evaluate or judge how well their analytical systems are operating. And best of all they will be comparing themselves against a system established by all of their peers.

Diagramatic Relationship

Between

Essential Elements in the National Conference


Discovery of Medical Needs.
Establish System for Discovery.
Materials & Methods
Establish Levels of
Absoluteness.
Establish Precision and Accuracy for
Analyte. Recommend level of absoluteness in
materials & methods necessary to meet analyte
objective.
Develop communication (organizational) system
for evaluation, approval and dissemination of
reference materials and methods.

ANALYTICAL GOALS AND QUALITY REQUIREMENTS

M. Desmond Burke, M.D.

Analytical goals depend, in an immediate sense, on the needs of clinical decision-making; ultimately, the clinical effectiveness of those decisions will be the determining factor for society. Thus, development of quality performance standards for reference materials and methods in clinical chemistry requires: (1) Analysis of useful clinical decision-making processes with development of an integrated theoretical basis for defining analytical goals in terms of clinical needs; (2) Translation of theory to practice with development of specific guidelines geared to useful clinical decisions.

Theoretical Basis

Physicians use analytical test results as follows: (1) to detect clinically inapparent disease (screening, profiling); (2) to confirm clinical evidence of disease; (3) to exclude disease in the presence of minimal clinical symptomatology; and (4) to assess prognosis in response to therapy.

Clinical sensitivity, clinical specificity, and prevalence of disease determine diagnostic validity. For any analytical test result, the degree of overlap in values between two subgroups of a population being tested is a function of analytical and biological variance. Reduction of analytical variance to a fraction of biological variance is an obvious analytical goal. For detection of disease, analytic variance should be reduced to a fraction of inter-individual biological variance; confirmation testing, attempts to exclude disease and trend analysis demand that analytical variance be reduced to a fraction of intra-individual biological variation.

The "cut-off point", "operating position" or "discrimination value" chosen to distinguish disease from non-disease or to confirm clinical findings or to exclude disease should be such that maximal information is provided at minimal cost. As a rule, sensitivity is important for detection of disease and is vital for the exclusion of

disease. Specificity is required for confirmation of findings.

A material or method must meet, not only the standards required for diagnostic validity, but also those of clinical usefulness: organized attempts to detect disease for the sake of detection, with little likelihood of influencing a favorable outcome, should be discouraged. The same applies to differential diagnosis for the sake of noting differences or to trend analysis for similar reasons [except for research purposes - Ed.].

Implementation Guidelines

1. Analytes should be adequately defined.

2. Analytical variance should be reduced to fractions of intra- and inter-individual biological variation consistent with current information.

3. Further attempts should be made to define biological variance under physiological and pathophysiological conditions.

4. The contribution of preinstrumental variation should be determined for each analyte considered.

5. Attempts should be made to determine the effect of inaccuracy on the indices of diagnostic validity.

6. Clinical decision-making should be subjected to more intensive study. Discrimination values that serve to confirm or exclude pathophysiological conditions should be determined that will not only maximize information but minimize costs -- both financial and clinical. Standards of analytical performance should be developed, not only at the "cut-off" between disease and non-disease but also at other critical decision levels.

ORGANIZATIONAL COMPONENT

Rex B. Conn, M.D.

The following statement has been approved by the Board of Directors of the American Scoiety of Clinical Pathologists, August 6, 1977.

General Objectives For The Conference

The ASCP is in general agreement with the stated objectives for the Conference. Coordination of efforts to develop an organization for communication and cooperation between professional societies, industry, and federal government is a desirable objective. Establishing appropriate specifications for reference materials and consensus agreement on the most appropriate definitive methods and reference methods are desirable objectives and can be accomplished only through a concerted, cooperative effort.

The ASCP believes that the objectives of the Conference can be accomplished most effectively by clear definition of our ultimate objective: improvement in patient care through more prompt and precise medical diagnosis. It is in the context of this overall objective that the ASCP submits the following considerations for discussion.

1) Science is a process, not a condition

All sciences, including clinical chemistry, must be in a state of continuing change if progress is to occur. It appears critically important that the current state of the art in laboratory methods and materials be considered as a starting point for further improvement rather than a condition which should be universally accepted by all laboratory scientists.

2) Continuation of successful programs

The most effective (and economical) approach to accomplishing the stated objectives would be to build upon existing programs. The activities of the Center for Disease Control in proficiency testing and licensure, the activities of the

National Bureau of Standards in certifying and providing laboratory standards, the College of American Pathologists' Inspection and Accreditation Program and proficiency surveys, and the educational programs of the American Society of Clinical Pathologists are examples of existing programs that might be better coordinated.

3) Materials and methods to be included

Chemical methods are utilized in many areas of the clinical laboratory and it might be undesirable to limit any proposed program to clinical chemistry per se. The stated objectives and methods for accomplishing them might be applied to laboratory procedures such as hemoglobin measurement, identification of bacteria by gas chromatography, various immunochemical procedures, and others. It is not proposed that participation in the Conference be expanded; however, it is hoped that any action taken would leave open the possibility for inclusion of all appropriate diagnostic methods.

4) Definition of "clinical laboratory"

In order to accomplish the broader objective, improvement in patient care, it will be necessary to provide wide dispersion of information about materials and methods for the clinical laboratory. The laboratories involved are a heterogeneous group, ranging from the 60,000 or so laboratories in physicians' offices to the approximately 6,000 hospital-based laboratories [and about 5,000 independent laboratories and several thousand public health and government laboratories - Ed.]. Methods for dissemination of information, and perhaps instruction of laboratory personnel, will be an important step in accomplishing the objectives of the Conference.

5) Avoidance of duplication of effort

In the interest of economy and organizational efficiency, it will be important to define the objectives and activities for each governmental agency, non-governmental group, or other organizational entity, to avoid duplication of effort and expenditure.

Possible Activities

The concept of a nationwide system for developing reference materials, providing these materials to the numerous laboratories that will use them, obtaining general agreement on what constitutes a definitive method and a reference method, and establishing lines of communication and cooperation should be welcomed by all clinical laboratory scientists and physicians who use clinical laboratories. The scope of activities that might be included in such a program is so broad that financial constraints will make it necessary to formulate careful plans and set priorities for development of a comprehensive program. The following activities might be considered in formulating plans for such a program:

1) Information exchange

Although scientific journals provide an effective method for communicating scientific observations, the information generated through this program will present unique problems in publication. Publication in most scientific journals is a lengthy process and would inordinately delay dissemination of information. Also, each scientific journal has a limited readership, usually determined by society membership, and use of such journals would result in undesirably distribution of information. For these reasons, it is suggested that a newsletter be established. Consideration should be given to publication of an annual update on current information and a directory of sources of reference materials and laboratory services.

2) Coordinating the availability of laboratory services

Every clinical laboratory in the process of establishing a new method requires some method of validating the results obtained. Similarly, manufacturers of reference materials, reagents, and kits need some (preferably independent) method of validating product specifications. A directory of clinical laboratories willing to perform analyses by definitive methods or reference methods on a fee-for-service basis would be of assistance to numerous groups involved in this program.

3) Method development and evaluation

Identification of inadequacies in presently available laboratory methods and reference materials will not be difficult. In contrast, facilitating the development of improved materials and methods will present numerous problems. Assistance to laboratory scientists in obtaining grant or contract support for the latter would be an extremely useful component within the overall program.

SUGGESTED ORGANIZATION

It is suggested that a coordinating committee be established with representation from appropriate governmental agencies, professional associations and industry. It is strongly recommended that membership on this committee not be exclusive and that organizations such as the American Medical Association, the American College of Physicians, and the American Society of Internal Medicine be represented. The committee would serve as an information exchange through a newsletter and perhaps an annual directory. The primary functions of the committee would be to identify objectives, set priorities, monitor progress, and coordinate activities of its constituent groups. It is suggested that ad hoc task forces be established when necessary to carry through specific tasks mandated by the coordinating committee.

An important organizational component will be financing the activities of the coordinating committee and of the task forces. An important early consideration will be whether such financing might come from a governmental grant, grants from private foundations, assessment of societies represented, or other sources.

If it appears advisable, a corporate entity might be established to provide an administrative structure for the program.

STANDARD MATERIALS

John A. Lott, Ph.D.

I. Need

One issue that this group must consider is the areas of need in clinical chemistry for calibrating materials. Once needs are defined, it will be possible to describe the kinds of calibrating materials that are required. In preparing this list I have kept in mind certain calibration materials that are available from the NBS as SRM's.

A. Protein standards for electrophoresis. There are really no calibrating materials available now and accuracy in this area is poor.

B. Triglyceride standard is needed. A single [agreed on Ed.] well-characterized fatty acid-glycerol ester is needed.

C. NADH standard. NADH that is commercially available is of uncertain purity, many contain inhibitors and unknown amounts of water. There is an urgent need here.

D. Enzyme standards. This may be utopian, but some start must be made toward standard enzyme preparations for all clinically important enzymes.

E. Direct (conjugated) bilirubin standard. There is a definite need for a standard here.

II. Definitions of Standard Materials

There should probably be no more than two defined kinds of standard materials:

A. Primary Standards. These are highly purified compounds with a known composition. The purity has been established by physical methods, e.g. melting point, specific gravity, isotope dilution-mass spectrometry, etc. In some instances, the purity is established by measurement of the absorbance of solutions of the analyte in a well-calibrated spectrophotometer. The purity and composition of the primary

standard is determined principally by physical methods, although chemical analysis may also be used. Primary standards are stable in the form prepared and maintain their composition during storage. The method of preparation of the primary standard has been demonstrated to give a consistently pure product of known composition.

Some examples of primary standards presently available are the NBS-SRM's, and certain commercially available "Primary Standard-ACS Grade" reagents: Both the NBS and the ACS have established criteria for their primary standard materials [Rand, R., What is a standard? Clin Chem 13, 55 (1967)]; [Meinke, WW, Standard reference materials for clinical measurements, Anal Chem 43, 28A (1971)].

B. Secondary Standards. All materials for which the composition is determined primarily by chemical analysis and comparison to a primary standard is a secondary standard. Thus a protein standard (e.g. human albumin) for calibration of biuret methods is a secondary standard, since the protein is analyzed by (e.g.) the Kjeldahl method for nitrogen and a factor is used. The ammonium sulfate used for standardizing the Kjeldahl method would be the primary standard. By this definition, control materials could serve as secondary standards if they have been analyzed by suitable reference methods and compared to primary standard materials.

III. Certification of primary standards

Any new primary material must be certified for content and stability prior to use. This is probably best done through the NBS, which also has the ability to distribute primary standards to users.

IV. Attributes of primary standards

In specifying purity, homogeneity, form, stability, etc., one must keep in mind the end use for the primary standard material. Conceivably, a primary standard could be prepared by mixing pure substance, e.g., proteins. For example, a mixed primary standard might be developed for serum protein electrophoresis.

Most primary standards would probably be "pure"

substances (e.g. 99.9% pure) and present in a stable form, usually in a dry form rather than in solution.

A very basic requirement of all primary standards is that they behave exactly like the analyte being observed. It must be possible to bring the primary standard into solution (usually) and in such a form that the matrix of the unknown and standard are the same or that the matrix effects of the unknown are abolished.

BIOLOGICAL REFERENCE MATERIALS

H. Peter Lehman, Ph.D.*

1. Definition

A Biological Reference Material (BRM) is one consisting of one or more analytes, in their purest available form, and contained in a matrix resembling, as closely as possible, the medium in which the analytes are measured in the clinical laboratory.

A BRM is a secondary standard in that the amount of constituent present must be determined as accurately as possible by a definitive or reference method of known reliability.

2. Purpose

The purpose of the BRM is to provide a specimen having an accurately known quantity of analyte (or analytes) of known purity in a physiological, or simulated physiological matrix for the purpose of: a) standardizing analytical method, (comparing routine laboratory procedures with definitive or reference methods), b) standardizing and/or calibrating instrument, (relating laboratory instrument performance and/or methodology to a definitive or reference instrumental analytical procedure), c) verifying quality control procedures, and d) evaluating candidate reference method or other analytical methods in their developmental stages.

The BRM may also be used as daily calibration materials or daily quality control material, but this is not the primary purpose of such materials.

3. Analyte

The analyte is the component to be measured. The component (compound) in the BRM should, as far as possible,

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be identical in composition and structure to the component in the physiological system. In the case of mixtures (e.g., 17-ketosteroids, total lipids, etc.) the amount of component in the BRM determined reference procedure should be related to a pure compound assayed by the same procedure (e.g., 17-ketosteroids) might be related to dehydroepiandrosterone (DHEA) by stating the amount of 17-ketosteroid equivalent to a given amount of DHEA in pure ethanol. In the case of enzymes that occur in isomeric forms, (e.g., LDH, CPK), the component is a mixture which should, through the reference procedure, be compared to the activity of a stated pure form of the enzyme, such as one (stated) isomeric form, e.g. total LDH activity in BRM is equivalent to a given activity of LDH 1 in aqueous buffer (specified composition, concentration, and pH).

The selection of a compound as an analyte in a BRM should be determined by whether there is an accepted definitive or reference method.

The composition in a BRM should be determined by the level of interference of the analyte reference method by other compounds present. Examples of such interferences are phosphate with calcium determinations by atomic absorption spectrometry, sodium with potassium determinations by flame emission spectrometry, cross reactivity for antibodies in radioimmunoassays of specific steroids, inhibition or activation of enzymes, etc. Quantities of possible interfering compounds in the BRM should be such that interference effects are so low that they have negligible effect on the accuracy of the reference methods for the analyte.

The degree of purity of the analyte source material will depend upon the type of material being assayed:

a) compounds which are available in the highest degree of purity (NBS certified standards), such as Na^{+}, Ca^{2+}, Cl^{-}, glucose, bilirubin, and cholesterol.

b) compounds which are available in high purity, but not as high as NBS standards, such as steroids, lipids, and vitamins.

c) compounds which are available in a "purified" form but with an unknown degree of purity, such as enzymes and other proteins.

For each BRM a decision will have to be made, depending upon the availability and purity of single analytes, as to whether the preparation should involve addition of analyte source material of certified purity to a physiological matrix or purification of the compound within a matrix.

The amount of analyte in a BRM should reflect the important clinical decision levels of the analytes in medical practice. Since the major purpose of the materials is to standardize a clinical laboratory procedure or instrument, the most critical region for standardization would be at the level where clinical decisions are made. For most assays the critical levels are those at the limits of the "normal range," where a medical decision to act must be made on the basis of a test result. For others they may be at a still higher or lower level involving some other diagnostic or therapeutic decision. For some assays, where treatment and therapy is dependent upon, or monitored by, small changes in physiological levels, the clinical decision level may be represented by a range. Thus, for some analytes a single BRM may be sufficient, for others several concentrations of BRMs containing different levels of the analyte may be desirable.

4. The Matrix

The matrix is the milieu in which the analyte is contained. It therefore consists of all other components of the system, including other analytes not being assayed by the specified procedure.

The matrix should resemble as closely as possible the milieu in which the analyte is contained in the physiological state, or that in which the analyte is assayed in the clinical laboratory, if this is different. Thus an analyte which is assayed in serum should be prepared in a BRM with a matrix resembling serum, even though in the physiological state the material is contained in slightly different medium, i.e. in whole blood (plasma). It may be necessary, because of the presence of interfering compounds in the physiological matrix, to prepare the BRM in a simulated or purified

matrix.

Physical matrix effects are important in some instances, for example where specimens are aspirated directly into an analytical instrument (atomic absorption spectrometer), or where micro volumes of specimen are required for an analytical procedure. In these instances the difference between the viscosity and surface tension of an aqueous solution of an analyte and the same compound in its physiological environment may be significant. It is therefore important that the BRM have matrix physical properties closely resembling those of the physiological fluid. A suitable matrix for a serum analyte may be: a) human purified serum where all compounds which interfere, or may interfere, with the reference assay procedure have been removed, b) protein solutions prepared from purified human albumin or purified albumins of animal origin (e.g., bovine), or c) synthetic nonbiological compounds (polymers) which simulate the biological milieu in physical properties.

5. Additives

A BRM should be prepared in such a way that the reference analyte is contained in a medium which is as free as possible from all compounds which may interfere with the reference method. Preservatives or stabilizers may be added to such materials provided they have been shown to have no effect (inhibition or enhancement) on the reference assay procedure. All additives must be identified as to their source and purity.

6. Biological Activity

A BRM prepared from human material should, as far as possible, be sterile, i.e., be shown to be negative for infectious agents such as hepatitis-associated antigen. If absolute sterility is not possible, criteria for biological activity must be established, e.g., permissible bacteria bacterial counts (and how and when these should be determined), absence of hepatitis-associated antigen. Broad-spectrum antibiotics may be added to the BRM provided that they do not interfere with the analyte reference assay procedure. The method for checking biological activity must be defined, and added antibiotics must be identified as to

their source and chemical purity.

7. Preparation

Biological base material may be of human or animal origin. Material should be free from obvious interferences, such as lipemia or hemolysis, and, as far as possible, from biological hazards (infectious agents) and high concentrations of foreign agents (drugs, drug metabolites, toxic agents, etc.). Special BRMs may be prepared containing selected compound(s), (e.g., high in urin estriol, high in thyroid hormones) by separate collection of base materials (pool serum or urine) from selected clinical populations.

Depending upon the BRM being prepared, the base materials may have to be modified to obtain a BRM containing the analyte(s) at the desired concentration and, as far as possible, free from interfering compounds. Such modification may simply involve the addition of desired amounts of pure analyte(s) to the base material or more complex steps such as a purification process (dialysis, selective filtration, selective enzyme action, etc.) to remove one or more interfering substances, followed by addition (if necessary) of desired amounts of pure analyte(s). All procedures carried out in the preparation of the BRM (purifications, additions or analytes, preservatives, inhibitors, etc.) must be performed in such a way that homogeneity, in terms of concentrations of all constituents, is maintained throughout the solution.

8. Stability

The BRM should be prepared in such a form to ensure maximum stability of the constituents for as long as possible. The stability of the BRM should be increased by lyophilization or by storage at -20 C. Lyophilization is the preferred procedure because of longer stability and easier storage and distribution.

Precautions should be taken in the lyophilization process to ensure that: a) BRM constituents are stable during the procedure; b) the conditions are controlled to ensure that a solid residue remains, having a low moisture

content (stated tolerances), and one which is easily reconstituted; and c) on reconstitution with a given volume of high purity water the solution formed contains the BRM constituents and matrix in their desired forms.

Data on stability of different analytes under a variety of conditions should be available from manufacturers of quality control or calibration materials in biological matrices. The manufacturing procedure of a BRM should be based on such information and should include data on the initial accelerated decay processes which occur for many constituents. The stability (shelf-life) studies of product BRMs should be performed by the reference laboratories on material which has undergone the complete manufacturing process. The reference laboratories should assay the levels of analyte present in the BRM, and the stability of each BRM under different conditions such as lyophilized BRM stored at room temperature (25 C), stored refrigerated (4 C), and stored frozen (-20 C to -70 C). These studies should be carried out over a predetermined time span by one or more of the reference laboratories assaying the material by the reference procedure according to a set protocol. On the basis of the results obtained, tolerance limits and shelf life of the BRM should be determined for the different storage conditions and should be stated in the validation and certification data for the BRM.

9. Assignment of Values

Since a BRM is a secondary standard the label concentration(s) for analyte(s) depends on the accuracy and precision of the analytical procedure(s). Analysis for assignment of values should be carried out in a predetermined number of reference laboratories utilizing an agreed reference method of high precision and accuracy. The reference laboratories should have the necessary qualified personnel and instrumentation to be able to carry out the reference method reliably. A laboratory may be used as a reference laboratory for one or more analytes, or BRMs, depending upon the specialty and competence of the laboratory. The analyte in a BRM should be measured by a preselected, clearly defined, unambiguous procedure, utilizing reagents of the highest purity available (preferably from the same manufacturer and lot number), using

laboratory supplies (glassware, measuring devices, etc.) of a defined quality, and instruments which conform to defined performance characteristics. The protocol for the reference laboratories must define the acceptable precision of the procedure in the laboratory, the number of assays to be carried out by each laboratory, the time period for carrying out the assays, and the way of reporting results. The methods for statistical analysis of the combined data from the reference laboratories should be specified.

Where feasible, the concentration determined for an analyte in a BRM by the reference laboratories using reference methods mey be compared to the "true" level determined by a definitive method in a laboratory designated as a national reference laboratory, i.e., a laboratory having the necessary qualified personnel and instrumentation to perform a definitive analysis.

10. Certification and Validation

The final product BRM should be certified in such a way as to describe the material and its properties to the fullest. Label and certification information should include:

a) name(s) of analyte(s)

b) nature and source of matrix (e.g., human serum, human albumin, bovine albumin, urine, etc.)

c) concentration of analyte(s) in reconstituted material

d) preparative procedures - purification steps for matrix, names and purity of analyte(s) added, etc.

e) identity and specifications of all additives (stabilizers, antibiotics, etc.)

f) identity and maximum concentrations of compounds present that are known to interfere with the reference procedure

g) reference procedure(s) used to determine concentration of analyte(s)

h) definitive procedure used to determine analyte concentrations (if any)

i) names of reference laboratories and responsible personnel

j) stated, or referenced, statistical procedures used to determine analyte concentrations

k) decay characteristics of analyte(s)

l) manufacturing date

m) date of analysis by reference laboratories

n) storage requirements

o) stability and tolerance limits (shelf life)

p) manufacturer

q) name and address of person responsible for BRM certification.

11. Administration

BRMs should be manufactured and processed by industry according to criteria established by a National Reference Agency.

The National Reference Agency should be an organization that establishes criteria and standards for all reference materials and methods, validates the performance characteristics of the materials and methods, and acts as a national depository and distribution center for the materials. The National Reference Agency should also act as the depository for all the experimental data pertaining to reference methods and materials.

The agency may be one already in existence (e.g., NBS, CDC, NCCLS) or should be established within an existing agency. Professional societies (ASCP, AACC, CAP, etc.), government agencies (FDA, CDC, NBS), and private industry (manufacturers) should be represented in the agency

governance.

In the area of BRM s the National Reference Agency should:

a) determine the composition of a BRM (single analyte, multiple analytes)

b) select (appoint) reference laboratories

c) select the reference methods for the determination of analyte levels

d) establish the protocol for the analyses and statistical evaluation of the data

e) appoint subcommittees to develop one or more BRMs

f) collect and store all experimental data generated in the manufacturing and validation procedures for the BRMs

g) certify the BRMs after manufacture and establishment of data.

A BRM subcommittee should be comprised of clinical laboratory scientists, representatives from professional societies and national laboratories having known expertise in the analytes in the BRM, a statistician, and a representative of industry (manufacturer(s)).

A BRM subcommittee should:

a) determine the composition of the BRM (analyte, matrix, stabilizers, antibiotics, impurities, etc.)

b) determine the concentration(s) of analyte(s) in the BRM

c) specify manufacturing procedures

d) review the recommended reference method and modify it if necessary

e) review protocols for reference method and modify it if necessary

f) select reference laboratories

g) carry out statistical analysis of data, modified if necessary

h) report results to National Reference Agency

DEFINITIVE METHODS

(ASCP Position)

Definition

A definitive method provides the ultimate (L. "final") quantitation of an analyte, distinguishable from other methods by its greater accuracy, precision, recovery, sensitivity (lower limits of detection), and specificity (freedom from interferences other than the analyte in question).

Function

Definitive methods serve as benchmarks for the accuracy of reference and working methods (the latter used in clinical laboratories). In this sense, definitive methods are the top tier in a three-tiered system of methods; both reference methods and practical working methods may be linked to definitive methods by means of standard and biological reference materials (as defined elsewhere in this symposium). Measurement of calcium in serum shows the sequence of development and evaluation of all three levels of methodology: a definitive method based upon isotope dilution/mass spectrometry (1), a reference method based on atomic absorption spectrometry (2), evaluation of the reference method (3), and linkage of definitive or reference methods to routine methods in routine use (4-6). [References for this Section].

Characteristics

Analytical techniques used in definitive methods will rely primarily upon unambiguous physical properties of the analyte. The most characteristic property of an analyte is its mass, and techniques such as isotope-dilution/mass spectrometry (ID/MS) should be used when feasible. Separation techniques which combine solubility and mass of an analyte (e.g., gas-liquid chromatography/mass spectrometry (GC/MS), may approach ID/MS as definitive methods, especially for more complex analytes (e.g., steroids and drugs).

Techniques depending on other physical properties of the

analyte may be considered in development of definitive methods, but would require validation through other modifications of the same technique or through other independent methods. For example, a gas-liquid chromatographic separation might be incorporated into a definitive method if a second chromatographic method in which a different liquid phase is used is available for purposes of validating the first separation.

Development

Selection of an analyte for development of either a definitive or reference method should be based upon 1) relative importance of the analyte, either in frequency of measurement in practice (e.g. serum sodium or potassium) or in the significance of the result in practice, especially where no independent clinical validation of the result is available to the practitioner (e.g., measurement of drugs which do not produce early and unambiguous clinical signs of toxicity); 2) availability of well-characterized standards materials; and 3) availability of suitable analytical techniques.

Specification of requirements for performance should include 1) accuracy, 2) precision, 3) recovery, 4) sensitivity, and 5) specificity. Accuracy may have to be defined operationally by several independent groups, starting with the same standard materials. Specificity (relative freedom from interfering substances other than the analyte) should refer to those materials that may be found together with the analyte in clinical samples (e.g., carbohydrates other than glucose, in the case of a definitive method for glucose).

Selection of candidate methods for further development will be by consensus of participants using available preliminary data. The complexity and cost of definitive methods will limit their development and use to a small number of laboratories. To reduce costs of development, one laboratory group should have primary responsibility for developing the definitive method. At least two other independent laboratory groups (preferably with different instruments if available) should validate the proposed definitive mehtod to within previously agreed-upon tolerance

limits. For example, if the ID/MS method for calcium in serum has an accuracy of within 0.2% in the laboratory developing the method (1), the validating laboratories should be able to reproduce this accuracy to within 0.3-0.5%.

A least three laboratories should have responsibility for maintaining the definitive method for use in validating reference methods and materials. Unambiguous experimental protocols and periodic interlaboratory exchange of standards and biological reference materials will be required to maintain the definitive method.

Relationship of Definitive to Other Methods

A definitive method serves as a benchmark against which reference methods (and in some cases routine methods) will be measured. Accuracy is the primary criterion, and a candidate reference method ideally should perform to within the limits of analytical error inherent in the different techniques used for the definitive and reference methods. Persistent bias of a reference method as compared with a definitive method should stimulate searches for either more appropriate standard and biological reference materials or for an improved reference method (the definitive method being assumed to be accurate).

Previous work suggests that use of biological reference materials with a protein matrix may reduce the discrepancy between definitive and reference methods (6). The maintenance of these two classes of methods should include frequent assays of both aqueous standards and biological reference materials.

Suitable definitive methods do not currently exist for certain types of analytes (e.g., enzymes, polypeptide hormones); for such analytes, reference methods will provide the only validation of working methods.

Definitive methods, when available, can serve to stimulate improvement in clinical laboratory practice when linked to interlaboratory surveys (4).

Summary

Table 1 compares the three classes of methods. Although definitive methods are not designed for widespread use, they serve as the ultimate reference point for accuracy in clinical laboratory measurements. Linkage of working and reference methods to definitive methods by use of assayed biological materials used in interlaboratory surveys can potentially improve clinical laboratory pratice.

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TABLE 1. CATEGORIES OF METHODS

Type	Definitive	Reference	Working
Protocol	Single (at any one time with	Single (at any one time) with standardization	Multiple-subclassified by principle instrument reagents protocol calibration
Cost	High	High	Low-Moderate
Participating Laboratories	$N \geqslant 3$	$N \geqslant 20$	$N \geqslant 100$-2000 (by subclass)
Validation	Consensus	Comparison to definitive method	Linkage by standards and biological materials assayed by definitive and reference methods

REFERENCE METHODS

Bradley E. Copeland, M.D.

The following is a draft statement and has not been submitted to the Board of Directors of the American Society of Clinical Pathologists for their approval.

Objectives

The primary objective in defining reference methods is to establish stable baselines for the continuous medical decision-making processes which are termed "medical diagnosis" and "medical treatment" and in which analyses in are used the process of making these decisions. Continuous long-term high-quality and cost-effective patient care is the goal.

The National Reference System must be both stable and flexible. Protocols for the National Reference System should: 1) permit and recognize change, 2) allow new proposals, and 3) require periodic reexamination of established methods.

Needed Protocols include:

1. Protocol for establishment of a reference method

2. Protocol for establishment of a candidate reference method

3. Protocol for establishment of an equivalent reference method which would relate to the problems of small laboratories in small countries with tropical, hot, humid conditions.

4. Protocol for a proposed modification of an established reference method

Why Are Reference Methods Needed?

Reference methods are needed to maintain the connection between advances or changes in methodology and the medical heritage and the medical experience with a disease process.

Because of the desire to detect disease while it is asymptomatic and therefore curable or preventable, our affluent society can afford to provide laboratory investigations for this purpose. The automated instruments for multichannel and discrete analyses have adopted methods which require small size, preferably no deproteinization, and rapid processing capability. Significant bias has been demonstrated in these newer methods (cholesterol, bilirubin, calcium glucose, albumin, uric acid).

In addition, the automated multichannel or discrete analyzers depend on secondary lyophilized protein-containing reference materials whose concentration has been established by established reference methods, usually including a deproteinization step. In many instances the secondary reference materials were assigned label values by comparison with an original batch of material and not by direct comparison using a reference method. Thus whole generations of instruments were disconnected from reference method values. The resulting significant bias trends are notably evident in the College of American Pathologists Survey Reports.

Also, reports of screening normal populations have come into disrepute because of dubious findings, such as 60% of physicians with increased urea nitrogen and 30% of grade school children with hypercholesterolemia. A combination of systematic bias in methods coupled with poorly defined normal population values was the combined cause of these failures. Referral to reference methodology could have prevented many of these failures.

Both overdiagnosis of disease and underdiagnosis of disease is possible unless a strict alliance is maintained between medical identification of disease and the reference method for measuring the chemical constituent used as an indicator of the disease.

HENCE THE NEED FOR RUGGED, REPRODUCIBLE AND ACCURATE REFERENCE METHODS DESIGNED TO MAINTAIN STABILITY IN THE ONWARD RUSH OF MEDICAL DIAGNOSIS AND THERAPY BASED ON CHEMICAL CONCENTRATION MEASUREMENTS.

Preservation of the Medical Heritage

The purpose of a reference method is 1) to maintain a consistent baseline for medical decision-making; 2) to maintain an intact medical heritage -- that is, identification by clinical investigators of the relationship of disease to chemical concentration or activity and the cumulative experience of physicians who use the measurement of chemical concentration or activity to identify a disease and to follow its progress. The reference method promotes the transfer of medical experience from the clinical investigator in one geographic location to medical practitioners in all locations. It also makes it possible to compare concentration measurements by clinical investigators and by physicians without the confusion of systematic bias.

Because many diseases have a low incidence but are of very great significance to the individual who has the disease (parathyroid tumor, adrenal tumor), there is a valuable medical medical heritage of 1) patient symptoms, 2) association of disease with identification of abnormal concentration of metabolic products, and 3) the utilization of this experience to predict and cure new cases of the same disease. The reference method should prevent dislocation of the association of data relating concentrations and the presence of disease, due to the introduction of systematic bias and (or) nonspecificity and (or) specific interferences because of introduction of new methodology.

A similar medical heritage is associated with the symptoms and clinical course of patients with abnormalities of electrolyte and protein concentration.

Historical Background

From 1935 to 1954, the reference method for glucose was the Benedict modification of the Folin Copper reduction-molybdic acid color-development method. This was established on the basis of its correspondence to glucose values for the same filtrate which had been incubated with yeast to remove glucose. The Benedict method showed considerably less reaction with non-glucose reducing substances than did the original Folin method. About 1955, the Benedict method was superseded by the Somogyi-Nelson method which combined the

protein-free filtrate devised by Somogyi in which zinc hydroxide is used as the protein precipitating agent with the sensitive copper reduction arsenomolybdate color development, which had been devised by Nelson. In the 1960's, the toluidine single-reagent method was proposed and established by the CDC as giving results equivalent to those of the Somogyi-Nelson method.

In 1975, the FDA proposed the CDC-developed hexokinase method which is currently considered the reference method for glucose. A successful study has been carried out by NBS verifying the absence of systematic bias between hexokinase and the definitive mass fragmentography method using deuterium-label glucose.

Returning to 1930, we can follow similar methods over the decades since then, and it probably would be useful to prepare a chart showing the reference methods as they have evolved. Some reference methods have shown remarkably little change; for example, the reference method for phosphorus in 1930 was the Fiske and SubbaRow method in which aminonaphtholsulfonic acid was used with the molybdate color developing reagent. This method has not been superseded, although there have been proposals for various different reducing agents and several color reagents.

The situation for creatinine is similar. The alkaline picrate method, first used in 1930, continues to be used in 1978. Here, the discussion was whether non-creatinine chromogens made a significant clinical difference, such that the creatinine procedure should include removal of the non-creatinine chromogens. After several studies it was concluded that removal of the non-creatinine chromogens had no significant clinical impact, and therefore this step could be omitted.

Cholesterol assay has an interesting history. In 1930, the method in most common use was the Bloor extraction method. F.W. Sunderman recognized that color development of the cholesterol ester did not correspond with color development of the free (unesterified) cholesterol. His studies indicated a successful way to obviate this problem of the time of color development so that use of a pure cholesterol standard gave a valid result for total

"REFERENCE METHODS"* IN THE U.S.A., 1925 - 1975

Glucose	--	Folin-Wu, Benedict, Somogyi-Nelson, o-toluidine, hexokinase
Urea nitrogen	--	Urease-Nesslers, diacetyl monoxime (linearized)
Chloride	--	Silver precipitate/back titration, Cotlove titration
Sodium	--	Uranyl zinc acetate (gravimetric), flame emission
Potassium	--	Platinum titration, flame emission
Bicarbonate	--	Van Slyke manometric, volumetric
Total protein	--	Kjeldahl digestion, and ammonia titration
Albumin	--	Salt fractionation, Tiselius electrophoresis, human albumin/bromcresol green
Bilirubin	--	Evelyn-Malloy; Bilirubin Recommendation, (Academy of Pediatrics, CAP, AACC) Jendrassik-Grof; NBS bilirubin SRM.
Cholesterol	--	Bloor extraction, Schoenheimer-Sperry, Abell-Kendall, cholesterol oxidase
Calcium	--	Oxalate precipitate permanganate titration, atomic absorption spectrometry
Phosphorus	--	Fiske and SubbaRow phosphomolybdate color
Creatinine	--	Alkaline picrate color development
Uric acid	--	Phosphotungstic acid; uricase spectrophotometric/(American Rheumatism Association).
Amylase	--	Somogyi saccharogenic

*[Note: Reference methods in the sense of "well-accepted" methods. Ed.]

cholesterol. Another method for eliminating the effect of esterified cholesterol was to hydrolyze the ester so that the cholesterol was all present in the free form. Schoenheimer and Sperry used this technique, coupling it with a digitonide precipitate, and this became the first recognized reference method.

It is interesting that the crystalline digitonide precipitate from the hydrolyzed sample on the washing assumed the consistency of toothpaste. When this was discussed with Dr. Warren Sperry, he confirmed the fact that the same change occurred in his hands as well. This change in the precipitate did not seem to affect the reproducibility of the duplicates or the analytical recovery of the material by this method. Nuances such as this are not usually recorded in the scientific literature, but should be. Another great change which has gone completely unnoticed was the significant shift found in cholesterol concentrations in human serum with the change from visual colorimetry to photoelectric photometry: an increase of as much as 300 mg/liter. Such significant changes due to technical changes must be recorded, now, as well as in the future.

In the 1940's, because the Schoenheimer-Sperry method is so laborious, the Abell-Kendall method was developed, which correlated very well with the Schoenheimer-Sperry. The hydrolysis is followed by an extraction step and the color is developed with acetic anhydride/sulfuric acid. It was and is utilized by the Framingham Heart Study in its fundamental work on the association of cholesterol with coronary artery disease. The Abell-Kendall method has continued to be the reference method, not only in the Framingham Heart Study, but also at the Center for Disease Control and NIH. At present there are two candidate reference methods: a) enzymatic-cholesterol esterase plus cholesterol oxidase, and b) high-performance liquid chromatography.

INFLUENCES OF CLINICAL INVESTIGATION

The previous section gives a good example of how the clinical research area plays a large role in establishing a reference method. In our conference, I see that there is a significant lack, in my opinion, of representatives of the clinical research area. Since it is in the clinical research

area that our major advances are made in the utilization of chemical measurements, it has always been my practice to ascertain, when looking for a reference method point, the current feeling among leading clinical researchers with respect to their selection of a reference method. The clinical research leaders will usually have very definite opinions as to which method is the most nearly accurate with respect to the compound involved, and they also have definite ideas about the precision of the method that they choose. Most important, they have made a determination of the relationship between the disease process and the methods available and therefore are aware of the relative problems of systematic bias.

On the other hand, we also know that the clinical investigator often does not have an absolute bias-free method, because his interest has been in changes rather than in absolute concentrations. In this second case, it is more difficult to transfer the clinical research information into clinical practice. I feel the clinical researchers need more communication and more consultation with us at the practical laboratory level so that they become more aware of the importance of quality control day-to-day systems universally used in medical laboratories and also of the importance of the concepts of the levels of accuracy and bias-free measurements which can make available to the practicing physician a more universally applicable and consistent diagnostic tool.

In summary, the clinical research area has a large contribution to make toward the understanding of which reference methods are justifiable and valid from the point of view of clinical research. Clinical research is the origin of the test used in patient care.

Among the other importance reference methods are those for calcium, bilirubin, total protein, and albumin. Certain clinical researchers play a very large role in setting the standards of practice with respect to utilization of certain tests--the Joslin Clinic, for example, affects glucose values used for screening diagnosis. Dr. Blackburn of the Cancer Research Institute at the Deaconess has influenced the use of serum albumin measurement in assessing the nutritional status of a patient; he believes that an albumin concentration of

less than 32 g/liter is a significantly low value. This may or may not be true. The importance of the point is that there are certain clinicians who, since they lecture widely and have achieved national recognition, play a large role in the utilization of laboratory measurements, and in my opinion, it would be a very useful thing to identify these individuals. In addition, the National Arthritis Association has a great interest in uric acid levels. Again, the definition of the inter-method variability in values for uric acid has been widespread, and we also know that the concept of its normal value in humans has had many different estimates with respect to male and female populations.

DEVELOPING TRENDS IN CHEMICAL CONCENTRATION ANALYSIS FOR DIAGNOSIS OF DISEASE

1914 - 1957	Discrete analysis of protein-free filtrates (to remove interfering compounds), with aqueous or aqueous solutions of pure compounds as standards.
1957 - 1967	Automation, with use of dialysis or direct methods to obviate protein precipitation. Small sample method proliferation. Development of day-to-day quality control (ASCP) and periodic national external quality control (CAP).
1957 - 1977	Secondary reference materials become the most frequent type of instrument calibrator. Secondary standards.
1957 - 1977	Widespread development of regional quality-control programs (day-to-day quality control with regional comparison). Transfer of definitive method technology by means of regional program quality-control serum pools.

Calibrators and Reference Methods

There are problems relating to secondary standards for reference methods. These secondary standards, which have emerged in the late 60's, are usually lyophilized materials, and until recently we found little difficulty in analyzing

each of the reference materials by using a reference method which had been shown to be valid when used with patient samples. We now have encountered several episodes where the reference material will not produce a clear protein-free filtrate -- in the case of phosphorus measurements probably due to an agent introduced to aid in solubilizing cholesterol.

TRANSFER OF TECHNOLOGY IN THE 1970's TO 1990's

Secondary Calibrators as a Transfer of Technology

As we have seen in the 1970's, it is possible to effect a widescale transfer of reference method technology via lyophilized reference materials.

The chief defect at present is that the producers of the reference materials have not systematically used reference methodology to standardize their products. Present FDA efforts to effect such regulations should be successful in removing the present defects that are the result of systematic bais.

Transfer of Definitive Method Technology

It is now theoretically possible to effect the transfer of definitive method technology by means of the large regional quality-control programs now extensively developed throughout the United States.

There are two current examples:

1) The Massachusetts Society of Pathologists Regional Quality Control -- Isotope Dilution-Mass Spectrography Calcium Definitive Method, U.S. National Bureau of Standards.

2) Seronorm -- Data Key Lyophilized Reference Material -- Nyegaard Co., Oslo: Na, K, chloride/neutron Activation Analysis; calcium/isotope dilution mass spectrometry.

Need for Flexibility

Another important thing to keep in mind is that once a

standard a chemical standard or a reference method is established, it seems inevitable that other investigators will be working on standards and methods which they will propose to supplant the established standards and methods.

Therefore, it seems particularly important to me that we establish a system which has the flexibility to accept change in an orderly fashion. This system necessarily must avoid disregarding new developments and the exploration of new concepts.

The methods for Hemoglobin, bilirubin, and the calcium Reference Method published by the NBS Committee have each undergone considerable discussion and, indeed, considerable change. The fact that there was no established authority permitted these changes to be made without difficulty, the only difficulty involved being that the new information indicated that new values were involved.

So, there is a great need to be sure that whatever system is developed has flexibility, is receptive to differing opinions and new information, and that it has the ability to record the scientific discussions as they proceed. This, I feel, is particularly important -- perhaps for the coming generation; the ability of our system to record discussion and observation about our present standards is the most important thing we will be doing.

ARCHIVAL STORAGE AND RETRIEVAL

Each generation will re-examine the work of the previous generation. If the figures are available, their conclusions can be clear. If the figures are not available, their conclusions, necessarily, will be incomplete. In my opinion it is our responsibility to leave complete records.

At the present time, however, I do not know of any organization which is available to keep such complete records. In proposing any organization, I would say that it should not be governmentally controlled -- nor should it be totally disconnected from the government -- and perhaps the NCCLS is a working model which might be used in this regard. The fact of the matter is that when any one of our national or international leaders in methodology, definitive methods,

or standardization now retires from practice, his expertise, his records, and his data are almost immediately unavailable and disappear from the scientific world. For example, where are Van Slyke's data now? Where are the data that Hiller and Plazin collected with Van Slyke? Not available! I think we must set up a mechanism whereby data on standardization, can be preserved.

The following leaders should be asked to leave their data to the USA National Archive: Castelli (Framingham Study); Sunderman, F. W. Sr.; Sperry; Kendall; Abell; Seligson; Gerald Cooper (CDC), Lipid Standardization Laboratory); Boutwell (CDC); Eavenson (FDA); Cawley; Fuller; Cali (NBS); Velapoldi (NBS); Mavrodineanu (NBS); Schwartz (NBS); National Heart Institute Diet and Drug Cardiac Studies; and USPHS Glucose Method Studies.

MEDICAL GOALS, FUTURE GOALS, STATE OF THE ART

With respect to goals, I believe that there is a large gap between desired goals, future goals, and state of the art. It is necessary that we set future goals and that we set desired goals, but it is also extremely important to keep track of where we are with respect to the state of the art. Inevitably, with respect to human endeavor, we all want be remembered in the best possible light, and in the area of analytical measurement this means high precision (low variability). The desired goals are often said to have been achieved on the basis of wishful thinking -- a single analyst doing all results in a single time period on aqueous materials. The state of the art, from the point of view of our point of reference, must be defined, in my opinion, in terms of multiple days and multiple operators and extend over a period of years. Methods which cannot give reproducible results under these conditions could be said to be unsatisfactory.

There is also a gap between the concept of using single-patient analysis with decision-making and replicate testing. If the physician wishes more reliability in any given result, he frequently will use replicate testing, and if a test lacks sufficient precision to depend on a single analysis, such as calcium, it is common practice to use multiple or sequential tests, or both, to achieve the desired diagnostic goals. In

my opinion, this important medical practice is not appreciated among those who are at present saying what are achievable goals and what are desirable goals. Also, it is not appreciated that for some tests the important thing is the day-to-day variability over a short term -- let's say within the 10 or 15-day stay in the hospital or at most a 30-day stay. This is particularly true of measurements such as sodium, potassium, carbon dioxide, and chloride. The physician is following day-to-day variables and he is following changes, rather than having any special interest in absolute values.

On the other hand, some tests are necessarily long-term tests, such as those for cholesterol and uric acid, where the long-term variation during six months to a year is particularly important. And there is a difference between variability over a short term and a long term, as has been demonstrated by the CAP surveys, where 30% of the variability is attributed to long-term variables, and 30% of the variability to short-term within-lab variables; 30% to the between-laboratory variables.

It has not usually been appreciated or acknowledged that the manufacturer also contributes to the variability between laboratories. It is important, as we have more and more prepared reagents, to begin to emphasize the fact that variability between lots is a frequent cause of increased variability within the laboratory. It seems to me that industry must begin to study what happens to their instruments after they leave the factory and consider that their responsibility does extend to an instrument giving consistent performance over a period of years.

Establishment of Goals

The following considerations are to be specified:

1) Purpose for which the test is used.

2) Decision concentrations requiring highest precision and accuracy.

3) Decision concentrations requiring less precision and accuracy.

4) Decision cutoff line as a range rather than a sharp boundary.

5) Capability of the instruments and methods available. A general principle is that a method or instrument should be utilized to produce optimum precision and accuracy. It should never be allowed to operate on an "anything goes" basis because the clinical use of the results may not require all the available precision.

Such requirements as 1) requirements for cleaning of glassware, which may occupy 50% of the time involved without any proven need, or 2) the excessively careful transfer of materials techniques, or 3) excessive statistical operations, or 4) calculations which require a least squares plot, all serve to inhibit the use of reference methods.

Therefore, I am proposing that there be candidate methods and that there be certain guidelines for evaluating candidate methods. I think that the criteria for a new method should be clear, and I think that the criteria for the work that must be done in order to support the proposal of a new method should be clear. It should also be emphasized with respect to secondary reference material or calibrators that it is necessary that these reference materials be similar to patients' samples so that preparation of protein-free filtrates for reference methods can be carried out. There seems to be an increasing number of methods in my experience where the secondary standard or reference materials, have properties which make them completely different or in large part different from the patient's serum. I've just now seen a 20 m mol/liter difference on sodium methods between flame and the specific ion methods, that appears to be entirely attributable to the lyophilized material used as a calibrator.

[In addition: The criteria for data submission would be specified. The methodology debates which occur during the establishment of reference methods are often acrimonious, supercilious, emotion-packed and excessively compulsive. (Personal communication.)]

The Practicing Physician

Finally, in the present conference there seems to be no opportunity for the practicing physician to be represented in the discussion. I would like to suggest that the following be requested to submit views, or at least be made aware of this conference: The American Medical Association's Committee on Scientific Affairs; The American College of Physicians; The American Diabetes Association; The American Heart Association; The American Rheumatism Society; The American College of Surgeons; The American Society of Internal Medicine; and The American Academy of General Practice.

In my opinion, we are talking about the practice of medicine and we are talking about medical matters in which members of the organizations just mentioned have a large interest and to which they can make an important contribution.

PROVISIONAL REFERENCE METHOD PRINCIPLES

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The pressure to develop and finally to adopt criteria for various levels of clinical chemistry methods has grown unabated. The thesis supporting these proposals is to improve the accuracy and precision of those chemical analyses that support and often guide clinical diagnosis, therapy, and management. Because so little data exist correlating the positive and negative relationship between diagnosis and clinical management on the one hand and clinical chemical accuracy on the other, one should have a healthy skepticism to the real success of these methodology proposals.

The entire question of sensitivity and specificity of a given test in assisting in the diagnosis of a pathological state has been applied only at best casually to a few conditions. A case in point is the diagnosis of diabetes. Most clinical chemists would agree that the current state of the art for determining blood glucose concentrations is very advanced. Review of survey data from the College of American Pathologists and The Center for Disease Control strongly support that contention. But has the improvement in glucose assays improved the diagnosis of diabetes? Obviously not, if one accepts the statements in 1975 taken from world authorities at the annual American Diabetes Association meeting. Those authorities gave a range for a postprandial glucose determination of 1.20 to 2.00 g/liter as being diagnostic of diabetes. If the clinical interpretation and diagnosis is based on such a range, a procedure that had a 20% coefficient of variation would seem more than adequate. However, glucose assays by any of a variety of methods easily have a coefficient of variation of 5 to 10%. Do we really need an official reference method for glucose?

Currently, a consensus exists that the hexokinase/-glucose-6-phosphate dehydrogenase-coupled method fulfills almost all the requirements for a reference method. That consensus was arrived through the process of publication, experimentation, and literature review. The clinical chemistry community arrived at this consensus without the need of a highly formalized system for granting approval.

Accepting the hypothesis that there exists a need to codify the procedures in establishing various levels of methodology standardization, the following comments are directed to the "lowest" level, the provisional method.

PROVISIONAL METHODS

Discussion about establishing criteria to define various levels or types of methodology have usually been confined to the classification of definitive and reference methods. Those methods are so highly defined and the procedure so rigorous that the best possible analytical results are obtained. Such methods should serve as a yardstick against which the performance of secondary, provisional, or recommended methods will be evaluated.

It shall be obvious to most clinical laboratories that these provisional methods are the ones that will fulfill the needs for reliable, sensitive, and specific methods adaptable to the clinical laboratory environment. In no way can the so-called reference or definitive method become part of the routine methods used for day to day measurements of patient samples. A casual look at the now accepted definitive method for calcium drives home the point. An analyst using a 10 ml sample requires two days of dedicated work to obtain a result by the neutron activation method for calcium. Even the proposed reference method using atomic absorption for calcium is a demanding technique.

The provisional, secondary, or recommended method (all three adjectives seem appropriate) is more than just a step behind a reference method in status. The recommended (I prefer this term) methods are to serve as a guideline to assist laboratories in implementing methods that will fulfill their service needs. There must be more than one recommended method for any analyte. Such recommended methods should be directed to various levels of laboratory activity. Automated, manual, and micro techniques should be part of the recommendations for any analyte. Therefore, it is conceivable that for a given analyte, several if not a dozen or more recommended methods will be acceptable.

The hazards of not taking the above approach can indeed be serious. If only one recommended method is established,

then all U.S. laboratories will be forced to use that method or face either revocation of license or refusal of payment for services. Moreover, establishment of only one recommended method would eventually give one manufacturer or supplier a windfall advantage. If one accepts the need for a library of recommended methods and for the system to have an impact toward improving laboratory accuracy and precision, there is a need for a coordinated effort in establishing appropriate criteria.

A recommended method should be based upon adequate experimental information. The working group should establish strict and well-defined protocols to include:

1. Reagent requirements

2. Control of instrumental performance

3. Preparation of standards and biological control materials

4. Must the proposed method be measured against an already-established reference method (if available)?

5. How much variation or bias from the reference method would be acceptable?

6. Size and composition of the population in which the analyte will be measured.

7. An evaluation of the effects of various pathological conditions, pharmaceuticals, diagnostic procedures, and even diet on the proposed method.

8. An expression of the sensitivity and specificity with respect to possible pathological conditions to which the proposed method applies.

9. The number of laboratories necessary to evaluate the method.

10. The review process used to reconcile discrepancies among laboratories.

11. The procedure used to permit submission of data to a repository for review by both participants in the evaluation and other interested parties.

12. Consideration of how the approving body will grant provisional status--by unanimous vote, majority, etc.

With the establishment of such a protocol, any experimenter, manufacturer, or chemist can proceed to elevate a proposed method to the status of a recommended method.

The recommended, provisional, or secondary methods are the day to day workhorse for the clinical laboratory. Those who have developed or found another approach that solves common or unique service problems should be given the opportunity to improve the status of that proposed method. The general rules established by the committee should be scientifically and technically demanding, but not administratively restrictive. For example, any mixture of laboratories--including hospitals, private, industrial, academic, and governmental--may embark on a program to evaluate a proposed technique and possibly elevate it to a recommended method. That procedure should be openly available to all, not confined to a few laboratories.

In summary, the criteria for a recommended or provisional method seems all important to the working situation of a clinical chemistry laboratory. If we are to maintain our current system of continued investigation and development of methodologies, a well-defined and acceptable approach to assure appropriate exposure of potentially valuable techniques is an absolute necessity.

COLLEGE OF AMERICAN PATHOLOGISTS

POSITION PAPER

The College of American Pathologists welcomes the opportunity to participate in this conference. The conference will provide an opportunity for the continuing interchange of views among the sponsors who share a common dedication to the advancement of the quality of clinical laboratory analysis in the United States. The conference will also serve as a forum for a reaffirmation of the basic strengths of the U. S. clinical laboratory measurement system -- a system that has led to the development of the sophisticated science practiced in clinical laboratories in the United States today.

Clinical laboratory science has grown rapidly and improved progressively during the past three decades. The changes are readily apparent to all who work in the field. The clinical laboratory of today bears little relation to the clinical laboratory of the 50's. The clinical laboratories of the 1970's may well be the largest applied measurement system in the history of man. Individual laboratories are now located in communities of all sizes throughout the country. Thousands of laboratories provide continuous analytic services, day and night, seven days each week. Compounds which were unknown to research scientists in the 50's are now measured routinely in clinical laboratories. Measurement of nanogram quantities of organic and inorganic analytes is now commonplace. Thousands of samples for esoteric analyses are shipped daily from one state to another and from one coast to the other in a vast referral network.

There has been a progressive evolution in the analytical methods that are used. New methods have appeared, many older methods have been discontinued. Complex technologies which were inconceivable in the 50's are now applied routinely. There is no evidence that the pace of this change is slackening. On the contrary, the introduction of the microcomputer may well herald the advent of a new era of sophistication.

The progressive development of the analytic capability has been complemented by the development of systems capable of monitoring the agreement of analytic results provided by clinical laboratories. These systems operate on a continuing basis and monitor trends in addition to measuring agreement at any one time. All published data from these systems show

progressively closer agreement of the results provided by clinical laboratories in successive years. Some analyses are now approaching a coefficient of variation of 1% among individual results provided by thousands of laboratories -- a remarkable achievement and a source of justified pride to the thousands of individual scientists who have made this possible.

The clinical laboratory of the 1970's plays a critical role in medical care. The role serves to reemphasize that clinical laboratory science is an applied science and that the goals of laboratory science must be continually guided by the needs of medical care. It is critically important that the conference retain this focus if the conference is to prove successful.

The goals of accuracy, specificity and precision are pragmatic goals which must be justified in terms of their clinical application. They are not abstractions that can be pursued beyond the point of diminishing return. The recent suggestion by Rogers[1] that "cost may prove to be the Achilles heel" of a successful health care system underscores the continuing need for this perspective.

The strength of the U. S. system lies in its pluralistic nature utilizing the resources of individual laboratory scientists, voluntary professional organizations, industry and the government in a spirit of constructive competition and enlightened self-interest. Each of these various groups is responsible for the development and the validation of their own activities. There is no overall defined strategy for allocation of responsibility. This framework has provided and can continue to provide the flexibility that is essential to continued and orderly evolution.

The strength of the U. S. system also lies in the sophistication of the U. S. marketplace. Myriad choices are made by thousands of individual scientists representing a variety of disciplines. The net result of these choices determines the overall quality of clinical laboratory work in the nation. These choices also determine the success of the commercial ventures which have been the source of so many technologic advances. None of these relationships have thus far been codified and this may be the most critical source of

strength.

The critical nature of this feature was summarized recently by Lord Hailsham comparing the English and American legal systems with the legal systems derived from the Napoleonic code; "More than anything else, perhaps, it is the uncodified state of American and English law that divides us from these systems. There are advantages and disadvantages in this. But what is quite clear is that so long as a system is not codified (that is, covered by statutes) it has the capacity to evolve without the restrictive shackles of past precedent ossified into immutable doctrine."[2]

This conference provides an opportunity for each of the sponsors to review their efforts to advance the quality of clinical laboratory work and the way they coordinate their activities with the other sponsoring organizations.

ROLE OF THE COLLEGE OF AMERICAN PATHOLOGISTS

The College of American Pathologists is committed by its constitution to work for the advancement of the quality of clinical laboratory work in the United States. CAP has taken an operational approach to this responsibility and has attempted to fulfill the charge by developing a variety of programs designed to both monitor and improve the quality of the work performed by individual laboratories. These programs have exerted a direct and an indirect effect on the clinical laboratory field. The programs currently provided by CAP in the areas of the laboratory under consideration at this conference include:

a) Survey Program (1949)

Circulates close to one million specimens each year for interlaboratory comparison. Approximately 9000 laboratories are currently enrolled in the program and more than 1,000,000 individual analytic results are processed annually in clinical chemistry alone.

b) Clinical Standards Solution (1952)

Provides working standard solutions for use as calibrators. Solutions are currently supplied for

16 analytes. The concentrations of the analytes are traceable to Standard Reference Materials from the National Bureau of Standards where these are available.

c) Clinical Standards Laboratory (1961)

Participates in the validation of CAP standard solutions and certifies commercial standards for cyanmethemoglobin, bilirubin and cholesterol.

d) Product Evaluation (1961)

Validates the claims of manufacturers for their laboratory products. To date claims have been verified for 35 products.

e) Inspection and Accreditation Program (1962)

Conducts on-site inspections of clinical laboratories and accredits the laboratories that meet standards designed to assure the quality of the work of the laboratory. The program is recognized as "equivalent" under the authority of CLIA '67. There are 1,376 laboratories currently accredited.

f) Quality Assurance Service (1971)

Provides data processing services for regional quality control programs. The QAS is used by 5 regional groups and one nationwide group. 850 individual laboratories submit data to the program. Individual subscribers submit data, monitoring as many as 50 analytic processes operated by the laboratory. The program currently calculates and accumulates data on more than 30,000 analytic processes operated by the subscribers.

g) L.P.A. Audits, Inc. (1976)

Derives market information for industrial subscribers from the CAP data base.

h) CALM (1977)

Documents equipment inventory, depreciation and monitors

equipment maintenance records.

CAP has coordinated its activities with the other sponsors of the conference and has maintained contact with all the participating organizations. These contacts can be summarized:

AACC and CAP joined in the development of the bilirubin standard and in the formation of NCCLS. CAP has worked with AACC and NBS in developing priorities for SRMs. Currently there is an active AACC/CAP liaison committee. CAP members and AACC members participate in liaison positions within the committee structure of both organizations.

ASCP and CAP fulfill complementary roles. Coordination is ensured through extensive cross-membership of individual committees. CAP concentrates on the operational aspects of the clinical laboratory, ASCP on the educational aspects.

NCCLS was formed following a realization in the CAP Standards Committee of the need for a consensus body for adoption of national standards. CAP put out the call for the first meeting of professional groups to found NCCLS. Members of CAP have participated actively as officers and in the area committees. It is CAP policy that standards developed within CAP will be forwarded to NCCLS for review and refinement before adoption as national consensus standards.

Industry is regarded as a valued partner by CAP in the search for advancement. The CAP Industry Liaison Committee has met regularly for many years. Many CAP programs would not be possible without extensive industrial involvement and the use of the talent and technologies that are available in the industrial sector.

NBS and CAP first worked together in the 1950's when CAP requested help in the intercomparison of high resolution spectrophotometers needed for the certification of cyanmethemoglobin. CAP worked with NBS on the development of bilirubin standards. A conference sponsored by CAP identified the needs for a cholesterol standard and developed specifications jointly with AACC that led to the cholesterol SRM. CAP actively supports the NBS development of definitive analysis and works closely with NBS in the field of

radionuclides.

CDC utilized advice from CAP in developing realistic requirements for independent laboratories when CLIA '67 was first implemented. In the past few years the principal involvement between CAP and CDC has been the challenge to make the concept of "equivalency" into a realistic mechanism for coordinating the activities of the voluntary sector with the needs of government.

FDA has had little direct contact with CAP. CAP looks forward to bringing scientific expertise and aggregate information from CAP data to help FDA resolve the dilemmas that the agency now faces as it attempts to implement legislation.

ANALYTIC GOALS

CAP Philosophy

CAP places a high priority on the development of analytic goals which can be used to relate the quantitative output of the clinical laboratory to the needs of medical care. The development of these goals is a difficult and complex undertaking. When goals are ultimately accepted as consensus standards they will exert a profound effect on all aspects of the clinical laboratory field. CAP believes the development of goals must proceed cautiously with ample opportunity for widespread review before any attempt is made to finalize either the goals or the statistical matrix in which the goals are to be applied.

It is also important that the goals be coherent from one laboratory discipline to another and that lessons learned in one discipline will be applied equally to other disciplines.

CAP Involvement

CAP has been actively involved in the development of goals through a number of committee activities. The paper published by Barnett from the CAP Standards Committee was a milestone.[3] The paper presented the premise that if goals are to be useful they must consider the context of clinical application and cannot be based on statistical formulae

alone. This premise is now universally accepted. CAP has sponsored a series of conferences on Analytic Goals in Aspen, Colorado. The first conference entitled Medical Usefulness in Microbiology was held in August 1975; the second conference on Analytic Goals in Clinical Chemistry was held in August 1976, and the third conference discussing Goals in Hematology was held in August 1977. The proceedings of the first two conferences have been published.

Each of these conferences attempted to synthesize the views of many individuals and to allow discussion among scientists from all sectors of the clinical laboratory field. The conclusions of the second conference have provided the basis for many of the concepts used in developing the outline for the present meeting.

Application of Goals to CAP Programs

The goals will have a far reaching effect on the individual CAP programs and on other comparable programs in the clinical laboratory field. Many CAP programs operate primarily on a basis of peer group comparison. Performance is related to the performance of peers and not to an absolute standard, thus the interlaboratory Survey program assesses individual analytic values by comparison with the distribution of values reported by peers; the QAS program compares individual laboratory statistics measuring long term analytic variance and long term bias between laboratories; the Product Evaluation Program confirms, or fails to confirm, manufacturer's claims, but does not judge them. The Inspection and Accreditation Program requires that long term analytic agreement is monitored but sets no standards for the closeness of the agreement. Each of these programs is evolving towards a system related to analytic goals but the pace of evolution is slow. It is also apparent as each evolves that the applications of the concepts of goals will differ for each type of activity, and the central philosophy will require adaptation to each type of program.

The CAP believes it essential that the pace remain slow. Premature attempts to apply goal based concepts would be counter-productive. Orderly progress must be based on documentation of the present norms of performance and a complete understanding of the theoretical basis for the goal

setting process. The CAP data base is fulfilling the first of these two requirements. This Conference can help provide a major step forward for the second requirement, an understanding and an acceptance of the theoretical basis for goal setting in clinical chemistry.

ORGANIZATIONAL COMPONENT

The CAP considers it essential that the clinical laboratory measurement system continue to evolve. It is equally important that the evolution build on the strengths of the present system and address clearly identified problems. The CAP does not believe that there is a demonstrated need for radical new initiatives.

The laboratory field has entered a new era during the past decade through the establishment of widely available data that make it possible to evaluate many facets of routine laboratory performance and to assess critically both trends and problems. These data must be the starting point for identifying problems and developing solutions. The existence of the data also provides a continuing opportunity to re-evaluate basic strategies and the basic requirements that are needed to ensure that clinical laboratory measurements are effectively meeting the needs of medical care.

It is critically important that the data be used to fulfill this role. It is no longer acceptable to reiterate dogma or develop theoretical strategies appropriate to the early decades of the twentieth century, which may have little relevance to the current world of computers, communications and sophisticated manufacturing technology. The challenge that we face today is to fully utilize the exciting potential of the technology that is now available -- a technology and an opportunity that would have appeared inconceivable 25 short years ago.

Survey

The picture that emerges from the CAP Survey data is one of progressively closer agreement from one year to the next among quantitative results in clinical chemistry.[4] The data show that the dispersion among results stems more from problems of imprecision than from problems of inacuracy.[5,6]

The implications of the data are clear. Method selection is critical and method precision must be a major concern in method selection and future method development. The accuracy of a method cannot be disregarded but is of less importance. Data are not available describing specificity and this may or may not be an important consideration. These conclusions are based on results submitted by participants to the CAP Survey program. The participants perform an estimated 65% of all laboratory analyses in the United States.[7]

These data are not presented to attempt to say that there are no problems. The field of Clinical Chemistry is expanding and as new analyses become feasible they can be expected to present problems. The data suggest that as these problems are identified they will be resolved by continuing application of the resources of our present system and our growing sophistication in applying these resources.

STANDARD MATERIALS

A sample of a pure standard material provides the foundation of a measurement system. The needs of the clinical laboratory field have been well supplied by the National Bureau of Standards. SRMs provided by NBS have justifiably become the accepted benchmark throughout the world.

The CAP has actively encouraged the development of SRMs and has participated in setting the priorities for development of further SRMs through the NBS Advisory Committee.

SRMs cannot fulfill the daily needs for standards in a routine working laboratory. The CAP has contributed to this need by distributing clinical standard solutions. The concentration of the analytes in these solutions is traceable to standard reference material from NBS when available.

Practitioners in the field face an unresolved dilemma when they attempt to apply values based on pure standards in a defined matrix to the complexities of human serum. The conference should consider the role of standards in an incompletely defined biologic matrix. Can the concentration values assigned to such material be readily transferred to

patient derived material? Can the values be related from one method to another? The CAP has, in general, believed that potential analytic interference and potential instability suggest that a continuously renewed source of materials with differing concentrations may be preferable to a single material.

The College also wishes to stress that useful data is provided for many analyses which lack standard materials both now and in the foreseeable future. The goals for these analyses must be for long term consistency within a laboratory and for agreement among the results produced by different laboratories. Such agreement should ideally be based on interchangeable values provided in common units. Data can still be useful when based on differing units with a conversion factor.

DEFINITIVE METHODS AND REFERENCE METHODS

The development of definitive methods at the National Bureau of Standards has added a new dimension to measurement in clinical laboratories. Definitive method technology now makes it possible to establish a value for the concentration of some analytes which is independent of the matrix and can be accepted by all scientists as a fixed point of reference for measuring accuracy.

The CAP believes that definitive analyses for inorganic cations and anions which have been developed are capable of providing values with sufficient accuracy to meet the needs of medical care and that these definitive values can be accepted as benchmarks. CAP looks forward to the presentation and critical evaluation of definitive methods for organic analytes.

CAP hopes that efforts will be continued to establish a second World Center capable of performing definitive analyses. Such a center should ideally operate independently and develop its own technology. The European Community would appear an ideal site. The need for a second Center will become more apparent as definitive methods are developed which attempt to measure the concentration of the potentially unstable organic analytes which comprise the bulk of the work in clinical chemistry laboratories.

The CAP believes that this conference can fulfill a valuable function through discussion of guidelines for the application of definitive methods so that the technology can be effectively applied.

Definitive methods offer a unique opportunity to measure the magnitude of inaccuracy. This capability can be applied to measuring the size of inaccuracy of reference methods; of routine methods; and of the routine work of clinical laboratories. These measurements provide an opportunity to assess the impact of inaccuracy on the clinical application of analytic values provided by routine laboratories. These various functions can frequently be combined so that a single definitive analysis performed at NBS can be used for one or more of these purposes.

The cooperative study conducted by NBS and CAP in 1975 fulfilled each of these functions.[6] A series of 12 samples were analyzed for 7 analytes. The data provide measurements of the accuracy of the routine work and routine methods used by the participating laboratories. Samples of the 12 specimens were incorporated into a multi-nation study of a proposed calcium reference method in Europe under the direction of Dr. S. S. Brown. The study also permitted some international participants in the CAP Survey program to compare their values to definitive values. Two samples from the series were circulated to laboratories enrolled in the Instand program in Germany through the cooperation of Drs. Merten and Boroviceny.

Approximately a hundred laboratories in Australia received all 12 specimens through the cooperation of the Royal College of Pathologists of Australia coordinated by Dr. R. G. Thomas.

The study also included a system of duplicates and ratio specimens which provided a confirmation of the repeatability over a six month period of the definitive values provided by NBS.

The CAP believes that this type of multipurpose study provides the most effective utilization of definitive value technology. It is hoped that similar studies can be conducted in the future, as further definitive methods are

developed for additional analytes. Further studies may also be warranted to evaluate the effectiveness of corrective action directed at resolving problems identified in the 1975 study.

The CAP believes that the application of definitive values to routine laboratory purposes is limited because of the cost and complexity of definitive value technology and the time constraints required for routine application.

The CAP has reservations about the value of the WHO serum project utilizing definitive values. The needs can be fulfilled far more readily and with far less expense through the type of mechanism conducted by Professor Whitehead and based on the English quality control scheme. The CAP Survey program, or any other large interlaboratory comparison program, could provide similar materials.

The interlaboratory Survey based materials would offer the substantial advantage of being distributed within a short time from validation and being continually renewed so that long term stability would no longer be critical.

REFERENCE METHODS

It is important that the conference clarify the role of the reference method. The concept of a reference method is a well established tradition and such methods have played a valuable role in advancing measurement science. In its traditional role the reference method was selected from a series of candidate methods. It was then described in sufficient detail to permit comparable performance in a number of different laboratories. It was frequently adopted by a sponsoring organization. Neither the reference method itself nor its potential application were defined by statute and any tendency towards rigidity was minimal.

This situation is now radically altered. The reference method is at the crossroads of many of the regulatory pressures in the clinical laboratory. Reference methods could become the first component of the measuring system to become official U. S. policy through publication in the Federal Register.

It has been suggested that reference methods must be documented by comparison to definitive methods, and hierarchies have been proposed to fix the relationship of each component in the measuring system. These changes have substantially lessened the chances for any further evolution in the designation of a reference method. The establishment of a reference method has become a costly undertaking which can probably be supported only with government funds. It is important that this conference ask whether there are problems in the clinical laboratory field of sufficient magnitude to warrant this massive effort. The conference must carefully consider the limitations and costs of a federally established reference method technology incorporated into statute. The conference may also wish to consider whether there are practical alternatives which can meet the needs as perceived from the regulatory standpoint. The CAP believes that the approach can and should be far more pragmatic.

There are unique differences from one analyte to another. These differences are apparent in the pattern of method development, the number of alternative methods that are widely practiced, the challenge of the analysis itself and in the application of the analytic results to patient care. These differences are sufficiently marked to suggest that it may be a mistake to think in terms of a single immutable strategy that could be equally applicable to all the various analytes.

It is now possible to consider individual analytes on a case by case basis and utilize whichever of the components of a measuring system that appear most appropriate. This pragmatic approach has also become possible through the existence of a data base which provides an effective yardstick for identifying problems.

From this perspective it may be useful to attempt to define the circumstances in which a reference method would be useful.

The CAP would suggest that a reference method is valuable when inaccuracy has been demonstrated to be a significant problem in routine clinical practice and when the inaccuracy is detrimental to patient care. The documentation of inaccuracy can be provided by comparison to a definitive

method or by data based on intermethod comparison.

There is probably general agreement that glucose and thyroxine are good examples of analytes which meet these criteria; cholesterol and calcium are more doubtful contenders; potassium and lithium analyses do not meet these conditions.

For analytes such as glucose and thyroxine a reference method fulfills a useful function as a fixed point for comparative studies of method bias and as a reference point for applying target values to calibrators and quality control materials. There is a substantial difference between these two applications and they are not fulfilled with equal success.

A measurement of intermethod bias requires a series of comparison measurements covering a range of concentration. It may include a variety of specimens and possibly an examination of sources of interference. Results obtained by a reference method can be used successfully for this type of study. Two recent examples include a comparison of glucose methods [8] and an evaluation of the accuracy of calcium analysis [9] by participants in an interlaboratory Survey program.

There are different problems when a reference method is used to assign a target value to a calibrator or as a target value in an interlaboratory Survey. The reference method value includes a random component of error stemming from the imprecision of the reference method. This random component can too easily be large. The reference method value is also affected by any matrix interaction or interference by other analytes. Either or both of these sources of error may invalidate the usefulness of the reference method for this application.

In addition, from the standpoint of the individual, there is no satisfactory way to assign the reference method value to the individual method being used. If the method is reputed to provide results with a 2% positive bias, is it correct to assign a reference method value and then assume that patient derived material will be comparable to the matrix of the calibrator?

These concerns are not raised to refute a useful role for reference method, they are raised to suggest that reference methods are not a panacea for a measuring system. A successful reference method system should permit the technology of the reference method itself to evolve and should exert, at most, a negligible effect on the development of new routine methods. It is doubtful if a federally adopted system can fulfill either of these criteria.

It is instructive to view the continuously changing pattern of method popularity during the past decade. These progressive changes are a striking feature of the clinical laboratory field. This pattern of changing methods has led to the development of many different methods for analyses such as glucose. There are now many methods capable of providing values that will cluster within a narrow range of concentration with minimal intermethod bias.[9]

The availability of many different methods readily available in the different laboratories is a strength that can be used to assign target values for concentration by pooling results from the various laboratories and methods. Such a pooled value offers a substantial degree of certainty when the methods are widely practiced and when appropriate methods of calibration are widely used. The employment of numerous different methods minimizes the possibility of matrix interaction. The Conference may wish to consider whether an approach based on this principle may not provide a satisfactory alternative to a single designated reference method. This approach would retain flexibility and encourage progressive evolution.

The Conference should also consider whether there is need for a reference method for analytes such as potassium and lithium. The evidence suggests that these analyses are performed satisfactorily in routine laboratories and the inaccuracy of routine methods is negligible.[6]

The designation and acceptance of one or more analytic principles may fulfill the needs of clinical medicine for these and comparable analytes.

SUMMARY

These brief comments have attempted to summarize the views of the College of American Pathologists. The comments are distilled from many individual opinions and have been presented in general terms. The comments include three recurring themes, the current state of Clinical Laboratory practice; the role of data describing Clinical Laboratory performance; and the place of Clinical Laboratory regulation. If the Conference can bring mutual understanding to these three themes it will prove to be a uniquely valuable undertaking.

Clinical Laboratory practice is in a continuing dynamic state of evolution. The science on which it is based can be proud of many remarkable achievements. It is important that the Conference reaffirm this perspective as a starting point for discussion. This Conference is attempting to chart the future directions of a successful endeavor, not to revise the directions of a continuing failure. Such a reaffirmation is particularly appropriate following the publicity on the shortcomings of clinical laboratories generated by the slow passage of a Clinical Laboratory Improvement Act through the Congress. The continued focus on individual instances and a few studies of limited relevance may be an integral part of the political process. It cannot be accepted as an objective appraisal by a gathering of leading scientists such as the participants in this Conference.

Data describing the performance of Clinical Laboratories constitute a uniquely valuable resource. The Conference participants, as scientists, should welcome the availability of such a factual basis and utilize it fully in planning for the future. The data describe present peformance and can be used to define present problems. Such a quantitative definition provides the logical foundation for future plans. Such laboratory data also provide the language that relates the efforts of the Clinical Laboratory to the needs of medical care. This language is essential if the Conference is to continually ensure that recommendations can be justified in the terms of practical application. The discussion of analytic goals at the Conference will be a valuable aid in this context.

This Conference will serve a particularly valuable purpose if the sponsors who are concerned with regulation can bring a better understanding of their needs to the sponsors from the private sector. Concern about the inevitable rigidity of regulation is a recurring theme. It appears too often, from the perspective of the private sector, that regulation is justified on the basis of a 'worst case' argument, while the impact of the resulting legislation is inevitably 'all case', and may be only marginally successful in correcting the 'worst case'.

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CENTER FOR DISEASE CONTROL

POSITION PAPER

by

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I. INTRODUCTION

Since automated instrumentation was introduced into the clinical laboratory in the mid-1950's (1), increasing emphasis has been placed on reducing intralaboratory variation, to improve laboratory performance. However, in the last decade the trend at both the national (2-15) and international (16-22) levels has been to develop and implement a National Reference System for Clinical Chemistry (NRSCC) based on accuracy. The development of a reference method for serum glucose (23) represents a major effort in this area.

Several medical and analytical factors are responsible for the growing awareness of the need for a measurement system based on accuracy, rather than simply on precision.

1. Medical Factors

Because today's clinical laboratory is one of the essential components of a health-care delivery system, all laboratories must give the same result within acceptable limits for the same analyte from the same sample, independent of time and geographical location. If comparable results cannot be achieved among laboratories, serious diagnostic error or erroneous treatment may result. Clinicians are especially concerned with accuracy (rather than simply precision) in some situations that are listed by Barnett (23):

(a) "... [the need] to conform with published values when there are accepted levels for separating normal from diseased individuals.

(b) "... when there exists a physiologic relationship between two or more analytes in the same sample.

(c) "... when dosage of medication is predicated on the determined level of some blood constituent.

(d) "... when metabolic exchange studies serve as a guide to diagnosis and treatment."

2. Analytical Factors

Past experience indicates that plans for improvement of analytical precision among laboratories have been only partly successful because they have finite boundaries and often lead to less improvement than desired. Mitchell (22) stated that plans based entirely on improving precision are not totally acceptable for several reasons, which include:

(a) "Different techniques for measuring the same substance may produce widely different results with a high degree of precision.

(b) "Without a means of showing which results are inaccurate, it is difficult to persuade laboratory heads to accept one technique in preference to others. Thus, interlaboratory variation is perpetuated.

(c) "The rationale behind the use of an assay cannot be fully elucidated in all circumstances if there is no certainty that a particular substance and that alone is being measured.

(d) "The purist attitude with which a responsible scientist approaches his work cannot be satisfied."

II. ORGANIZATIONAL COMPONENT

1. Introduction

The basic purpose of this Conference is to develop an "UNDERSTANDING" of the nature of the component processes involved in developing, accepting, and utilizing reference materials and methods in clinical chemistry. The following concepts and elements of an effective National Reference System for Clinical Chemistry (NRSCC) are proposed to permit operation by consensus and with the least possible regimentation of participating individuals and groups of the scientific community, Government, or industry.

2. Presuppositions

Each organizational group participating in the

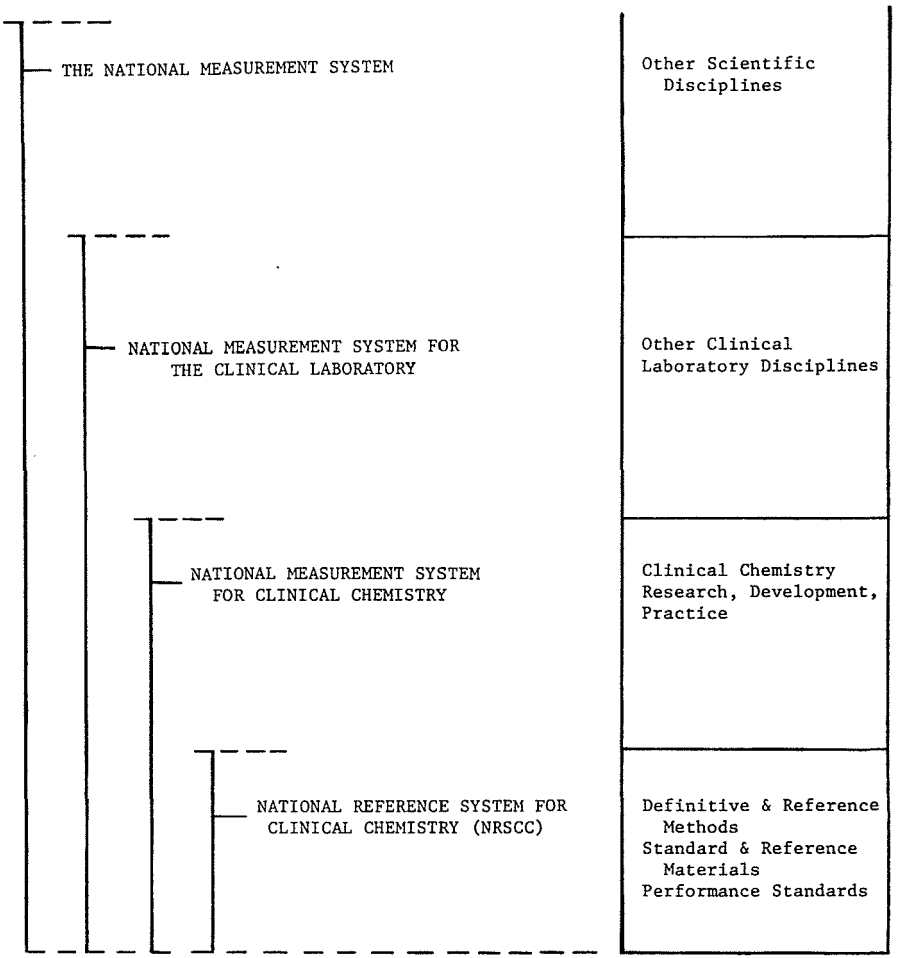

THE NATIONAL MEASUREMENT SYSTEM
NATIONAL MEASUREMENT SYSTEM FOR THE CLINICAL LABORATORY
NATIONAL MEASUREMENT SYSTEM FOR CLINICAL CHEMISTRY
NATIONAL REFERENCE SYSTEM FOR CLINICAL CHEMISTRY (NRSCC)
Other Scientific Disciplines
Other Clinical Laboratory Disciplines
Clinical Chemistry Research, Development, Practice
Definitive & Reference Methods
Standard & Reference Materials
Performance Standards

Figure 1. Interrelationships within the National Measurement System.

Conference brings with it some presuppositions, one of which is common knowledge. Other presumptions are unconscious or implicit, and still others are well understood and deemed desirable. In what follows, we describe the basic outline of the presuppositions which form the basis of the Understanding which the Center for Disease Control (CDC) regards as desirable.

Some of the functioning components of a National Reference System for Clinical Chemistry already exist, in the form of the many interested individuals and groups already participating in a series of loosely defined and insufficiently understood processes.

The proposed "National Reference System for Clinical Chemistry" would be a part of a larger conceptual system, "The National Measurement System for Clinical Chemistry," which would include such processes as the measurement and control operations conducted in clinical and clinically related laboratories of several types: service laboratories, research laboratories, industrial development laboratories and control laboratories. The National Reference and Measurement Systems for Clinical Chemistry, in turn, would be parts of another, still larger conceptual system, which might be termed the "National Measurement System for the Clinical Laboratory," which would be a conceptual part of a still larger "National Measurement System" (see Figure 1).

The current NRSCC has developed beyond the stage of infancy; it now needs wider understanding and guidance. Consequently, parental agreement in order to avoid conflicting or competing efforts and to derive and implement collaborative agreements among all groups affected by the system.

The present system by which reference materials and methods are developed, accepted, and applied has many fundamental advantages, not the least of which is that the talents and resources of many individuals and groups are utilized. Any understanding should preserve the opportunities and effectiveness so far demonstrated. Steps required to assure general acceptance of reference methods are more expensive and require more cooperative effort than has been generally understood. To utilize such resources

efficiently and to ensure their application to tasks of high priority and maximum yield is a difficult task.

3. Elements of a Voluntary Consensus Approach to a NRSCC

In addition to the presuppositions listed above, there are a few general principles which CDC believes should serve as guidelines in reaching necessary understandings. (For the sake of simplicity, the development of a National Reference Method is presented here to delineate the steps involved. Similar steps are implicit in the development and consensus recognition of national definitive methods, standard materials, and reference materials.

Flexibility

We believe that the National Reference System for Clinical Chemistry should not rely solely on Government agencies to carry out all development, validation testing, and transferability testing. Our concept, therefore, also includes the carrying out of these functions by private initiative as an essential element in dynamic interaction at all stages. (see Figure 2: Outline of System Proposal.) The various steps, including review appraisals and national consensus evaluations, would be the same no matter which approach is chosen.

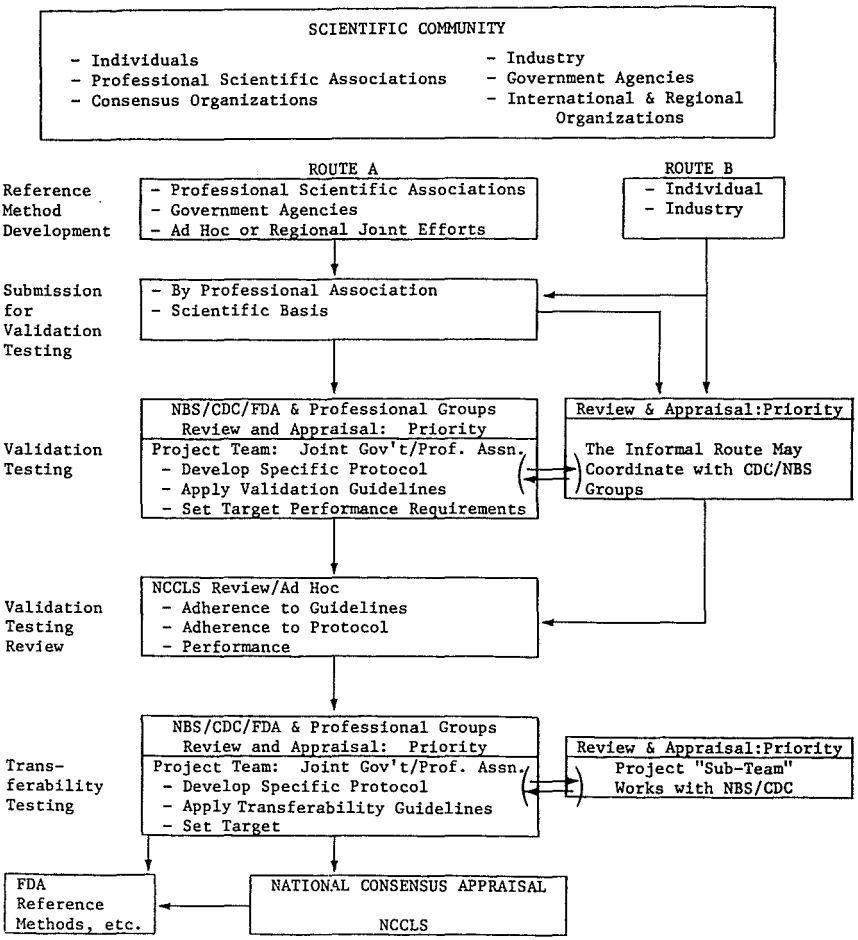
Acceptance

Since NRSCC Reference Methods must be widely accepted if they are to be effectively applied, their development and recognition require a national consensus and approval by some formal mechanism.

Formal Evaluation

Before they are submitted for consensus, proposed reference methods must be subjected to at least two formal evaluation procedures (validation testing and transferability testing) by basic criteria and procedures yet to be fully developed.

Figure 2: Outline of System Proposal



Developmental Studies

Methods of such critical importance should be developed along agreed-upon lines and should conform to predetermined criteria. To ensure that adequate scientific input is available and problems are worked out before the critical stage of validation, development of a candidate method should ideally be conducted under the sponsorship of cooperating groups having primary interests in the NRSCC. However, equally valuable work can often be done by individuals. The process consists of planning stages in which such factors are established or determined as: (a) priority; (b) precise definition of the analyte; (c) nature and size of sample needed and method of collecting and preserving it; (d) availability and nature of standard, reference, and control materials; (e) performance requirements such as accuracy, precision, specificity, cost, and productivity. This basic scientific and practical information and similar information on alternative analytical possibilities must first be acquired. The developmental stages include establishing the bases for verifying the analytical approach in meeting the target requirements, deriving the basic procedure, optimizing all variables, and describing the procedure in detail in written form.

Validation Testing

When a reference method is being evaluated for acceptance, it should be independently validated to assure (1) its conformity with the general guidelines in content and format, and (2) its accuracy, precision, specificity, and workability as a procedure. To ensure that the priorities are properly chosen to justify the large investments of effort, we propose that each candidate reference method be screened for scientific merit and priority by a Project Team consisting of the professional associations and the Governmental agencies which will allocate the task of Validation Testing.

The extensive resources required for comprehensive validation testing, which is most efficiently carried out in one laboratory, will be available in only a limited number of institutions. CDC regards validation testing as one of its prime responsibilities and would be (and is now) assigning a

high priority to this activity. However, the number of projects that any one institution can accommodate at one time is limited. Therefore, validation testing (according to Guidelines to be developed for such testing) is also envisaged as being open to private initiative and could be coordinated with testing performed by governmental Project Teams.

As a part of the required protocol, validation testing of a proposed reference method would include a conclusive and explicit comparison with any existing definitive method. Developing a means to make this comparison is an important aspect of the mission and responsibility of the National Bureau of Standards (NBS). Certain aspects of the proposed Reference Method are of signal importance to the mission of the Food and Drug Administration (FDA) in establishing limits of acceptability for accuracy and specificity of diagnostic products. Because of these governmental interests and responsibilities, the Project Team organized to develop the validation testing protocol will include representatives of NBS, CDC, FDA and other interested groups.

Transferability Testing

Transferability testing of a proposed and validated Reference Method should be undertaken after a separate and impartial review by a national mechanism to determine that the Validation Testing Guidelines were followed and the results were adequate to warrant the large effort required for Transferability Testing.

Transferability Testing should be conducted according to general Transferability Testing Guidelines (which must be developed and accepted promptly through a national consensus mechanism), and should follow a specific protocol developed by the Project Team responsible for the Transferability Testing. Each team would have representatives from the three governmental agencies and from the cooperating laboratories and others. CDC regards transferability testing also as one of its prime responsibilities and would be (as it is now) assigning a high priority to this activity.

4. Consensus Review

A suitably developed Proposed Reference Method (a) which has been validated by being tested in conformance with the guidelines, (b) which has been tested for transferability by a group of laboratories, and (c) which meets protocol-specified performance requirements should be ready for review on a national scale.

5. Transfer to the Scientific Community

Even after a developed, validated, and transferability-tested Reference Method (or Reference Material) has been approved through the national consensus mechanism, transferring it to the scientific community and implementing its beneficial effects on clinical laboratory performance will not be easy or automatic. The greatest impact of national reference bases will undoubtedly be felt through the development and dissemination of materials reflecting more substantial accuracy bases: "secondary" reference materials for evaluation of laboratory and product performance; calibration materials having common and more accurate bases for assigning concentration values; and reagents and other diagnostic products-all (in time) traceable to national reference materials and methods. Although the transition may be lengthy and may require readjustments by both laboratories and industry, the major effort will be required in developing the mechanisms for such transfer and in maintaining the traceability.

This responsibility will mainly devolve upon those who maintain and continue to use the reference methods at key points throughout the laboratory community so that continuing analytical validation is performed: (a) in clinical investigatory laboratories and industrial laboratories for research and development in analytical methodology; (b) in industry or its collaborating laboratories to relate performance and assigned values of their products to NRSCC accuracy bases; (c) in laboratories of agencies and professional groups evaluating or certifying clinical laboratory performance. Continuing programs (and resources) must be established to interact with these needs and assist these efforts, and here the missions and concerns of government agencies must play a part. For example, CDC has

realized the importance of this activity and has chosen several standardization programs and a "Manufacturer's Assistance Program" as the ways in which reference methods and associated materials may be effectively utilized by the scientific community. Professional groups must provide and initiate investigative programs to establish population norms for reference methods and must guide their membership in relating them to patient's values obtained with current routine procedures. Industry will have a key responsibility--and will bear a considerable share of the cost--in maintaining close congruity in product use among the reference bases and clinical laboratory results.

6. The Role of Information Exchange in the Working System

The proposed open, voluntary, unstructured framework of a National Reference System can work only if two essential elements are supplied: (a) cooperative participation of all groups, and (b) full, prompt, free communication of information. Existing elements of the NRSCC have been functional to the degree that participants have exhibited responsible communication and cooperation, but because this information has been in the past broadly disseminated only post hoc, confusion of purpose and intent and an enormous waste of time and effort have resulted. The consequent working at cross-purposes has slowed progress considerably.

In order to establish an open and extensive communication network within the NRSCC, we propose that the publication of the nature, extent, and current progress of activities in this area be assigned to some agency, public or private that can implement this function. The publisher should assume the responsibility for periodically interrogating appropriate individuals and groups, and the ensuing publications should be essentially in the authors' words and style with minor editorial refinements. This guideline seems necessary because of the great importance we place on individual and private group initiative and the important effect publication of current information on work plans and progress can have on similar efforts of other individuals or groups working in the same or similar areas.

7. Elements of the National Measurement System for Clinical Chemistry

Over the last 5 years a consensus has been developing as to the functional elements of the overall National Measurement System as applied to the needs of Clinical Chemistry. These elements are:

(1) definitive methods,

(2) standardized reference methods,

(3) routine service methods, which are empirically related to reference or definitive methods,

(4) certified standard materials and validated reference materials,

(5) routine or working calibrator materials,

(6) calibration processes as applied to definitive and to reference methods,

(7) evaluation and control materials and procedures of various types for routine quality assessment and maintenance, and

(8) performance standards that are based upon clinical requirements and measured in absolute terms.

Although this Conference is primarily concerned with the NRSCC, which relates reference methods and materials to standard materials and definitive methods, it must consider these elements in relation to routine methods and their calibrators in terms of standards of acceptable laboratory performance.

This Working Group is charged with exploring the organizational interactions that will most effectively implement desirable activities leading to the development and acceptance of reference methods and materials. These elements are currently being developed and tested by individuals and groups, singly or cooperatively. Our assigned task is to devise a cooperative arrangement whereby

our present efforts are more effectively managed, and yet to preserve those features of creative and scientific challenge are currently contributing to the development of the elements of the National Measurement System for Clinical Chemistry.

Individual Scientists

Innumerable published and unpublished contributions of individuals and groups will provide the scientific basis for the measurement system for any given analyte. For example, a chemist who is responsible for routine analysis of patients treated with psychotropic drugs may be in an advantageous position to detect chemical and physiologic drug interferences with a wide variety of analytes. These findings, in turn, contribute to the scientific understanding of methodological specificity and of practical requirements for sensitivity and accuracy of routine and reference methods and reference control materials.

Professional Scientific Associations

Such groups can assess the scientific needs of their professions on a priority basis. They provide the mechanism for arousing the concern, recruiting the technical competence, and for stimulating the scientific study and the consensus agreements required for a NRSCC.

Standard Setting Organizations

When the National Committee for Clinical Laboratory Standards (NCCLS) was formed, there was little nationally organized concern about and interest in standard-setting activities in the clinical laboratory. NCCLS now provides the forum in which standards may be proposed, their validity and consensus challenged and debated, and in which timely revisions may be effectively proposed and implemented after standards are adopted.

The function of NCCLS in determining whether and to what extent national consensus exists is essential to the review and adoption of any reference method laboriously developed in the way we have outlined. In the consensus process, careful distinction between identifying such consensus and special advocacy on the part of its members, its board, or its area

or subcommittee working groups will be necessary.

It is proposed that NCCLS be assigned a central role in two areas. The first is that they review proposed methods prior to their being accepted for Transferability Testing. This would ensure that Transferability Testing would proceed on the basis of a wide acceptance of need, priority, and scientific validity and hence would justify expending the time and resources required by such testing. For example, under this system, the CDC validation of the glucose hexokinase method would be subjected to NCCLS review prior to its use in Transferability Testing. NCCLS is also assigned its (self-defined) role of assessing consensus after Transferability Testing. When Transferability Testing has been completed to the satisfaction of the Project Team, the method will be submitted to the NCCLS for approval through its consensus process, as would any other standard. However, it should be noted that the consensus mechanism must provide for government agencies to meet their legally mandated responsibilities.

Industry

Since its establishment, industry has been engaged in standard setting in clinical chemistry. Marketing implies products with defined characteristics, and competition implies product differences. In order to be effective, the NRSCC must contribute to the capability of industry to provide well-defined products (instruments, reagents, diagnostic products, devices, reference and control materials, and recommended procedures) to consumers who are by now well informed and able to make critical judgements on the basis of their needs. Industrial scientists also contribute as individuals and as groups in developing the scientific base for the NRSCC and are involved in the consensus mechanism (NCCLS) in which compromises are reached in competing views.

Government Agencies

The Center for Disease Control (CDC) has a specific statutory mission to work toward improving all health laboratories, which for clinical chemistry requires the effective operation of the NRSCC. We recommend that CDC be

the lead agency within the government sector having primary responsibility for:

(1) developing, validating, and maintaining reference methods,

(2) developing, testing, and producing of the biological matrix-based reference materials that are essential for the validation and transfer of reference and definitive methods,

(3) designing, planning, conducting, and coordinating the Transferability Testing of reference methods in conjunction with NBS,

(4) training and education of designated personnel in the proper use of reference methods and materials.

As it has in other fields, the National Bureau of Standards (NBS) has a primary responsibility to provide the fundamental basis for measurement in clinical chemistry. It has a unique competency to develop and to test analytical procedures so exhaustively and so unambiguously that they can be classed as "definitive methods." NBS also undertakes the development of primary standard materials with defined content of given analytes. These are essential, not only for developing and testing the definitive methods, but also for developing and testing reference and routine methods.

The Food and Drug Administration (FDA) has a unique responsibility to assure that products used for diagnosis are safe and effective and meet standards of performance.

All governmental agencies are delegated by law to organize their program activities and their interactions with other governmental agencies in order to carry out their basic missions most effectively and economically. For example, CDC and FDA have an interagency agreement which defines certain related objectives in carrying out their respective responsibilities, including exchange of information, joint meetings, and CDC development of scientific advice on product labelling and product class standards. This program quite evidently has impact on the FDA's product evaluation and standards development.

The NBS and CDC have a long-standing agreement and have worked together in developing the definitive and reference methods for several analytes including calcium, glucose, electrolytes, cholesterol, and uric acid.

9. Summary

The continuing process of developing and maintaining any National Reference System must be flexible and easily modified as well as being more rapidly responsive to new knowledge and understanding than hitherto. This proposal is directed toward a collaborative system that seeks to minimize irreversible locking-in either of concepts or institutions into molds before "consensus" becomes universal agreement. This document, therefore, can only present a very rudimentary framework of an approach that is unusual in our highly organized society. The proposal is advanced to stimulate concepts and ideas that will crystallize our general understanding of what we have been doing, what we need to do, and how we should go about doing it.

The present system, or nonsystem, has resulted in some very concrete results. It is hoped that a better understanding of the various functions each of these groups is serving (some of them only incidentally targeted to their main purpose) will contribute to implementing a coherent National Reference System, and that less duplication of activities and more efficient application of relatively scarce scientific manpower will make all of our efforts more effective.

III. ANALYTICAL GOALS AND QUALITY REQUIREMENTS

1. Introduction

Developing a viable and functioning National Reference System for Clinical Chemistry (NRSCC) will depend upon the availability of (1) reference materials of uniformly high quality for evaluating laboratory performance and (2) analytical procedures (definitive and reference methods) used to assign quantitative values for their analyte concentrations. The quality and reliability of these tools are of direct concern to practicing clinical laboratories,

producers of diagnostic products, and groups and agencies which are responsible for promoting, certifying, or regulating quality in both areas. If national consensus reference methods are to become the "official arbiters" of acceptable laboratory methods and diagnostic products and if reference materials are to be the basis of evaluating acceptability of laboratory performance, they must provide accurate and reliable analytical information. Consequently, explicit and detailed analytical goals and performance specifications must be developed, defined, provided, and achieved for both the methods and materials as they are integrated in a functioning NRSCC.

2. Requirements

The primary task of the working group on Analytical Goals and Quality Requirements is to devise an approach to setting quantitative limits upon the bias, specificity, and precision of:

(1) assigning quantitative values to materials used to measure performance accuracy in the NRSCC;

(2) analytical methods used in establishing the accuracy base of these materials.

Consequently, the reliability requirements for reference methods and materials must satisfy three rigorous demands:

Elimination of Bias

Given a reference method capable of known accuracy, the system must be demonstrably immune to bias or drift from its original base. Rather extensive experience with transferable reference methods has shown the need for independent documentation and continued monitoring, not only of its successful transfer, but its maintenance in the reference laboratory. Such monitoring requires the availability of qualified reference materials. Parallel analyses of a high percentage of reference materials with the samples to be tested are required to verify maintenance of the accuracy base within 0.5% - 1.0%, a range representing the minimum detectable deviation from some reference point (e.g., a certified reference material or definitive assay) with the

best currently achievable precision for many assays.

Minimization of the Effects of Variability

If a method or product is to be evaluated by parallel analyses with a reference method, the imprecision of the reference method must either be low or be so minimized by replicate determinations that the uncertainty of the reference measurement is reduced to perhaps one-fifth of that of the performance of the test method or product. To achieve a desired confidence interval for estimates of a limiting value such as bias, the number of determinations required increases geometrically as the S.D. increases. Therefore, unless the C.V. of the reference method lies well within 1%, the analytical load for both assays quickly becomes intolerable.

Reference Method Productivity and Output

In situations involving parallel analyses, the reference method must provide an acceptable analytical through-put and must be accompanied by high analyst productivity. In addition, it must be usable by a fairly large number of laboratories of demonstrated capability.

3. Recommendation

The NRSCC is charged with the responsibility of interrelating and integrating performance standards for acceptable laboratory practice with performance standards of reference methods and materials. This will be a difficult and complex task; however, several pertinent subject matters have been previously discussed at the 1976 Aspen Conference on Analytical Goals in Clinical Chemistry, which was sponsored by the American College of Pathologists. We recommend that the Working Group on Analytical Goals and Quality Requirements use these proceedings as a starting point in beginning its task.

IV. MATERIALS

1. Introduction

If an accurate chemical measurement is to be made, an adequate quantity of the pure analyte to be measured must be available. The report of the Reference Materials Subcommittee of the Commission on Analytical Chemistry, Division of Chemistry and Technology, National Academy of Sciences-National Research Council of 1968 (25) indicated that the field most in need of such materials was clinical chemistry. Even though substantial progress has been made (2, 3, 5, 9, 12, 13, 22, 26-28), additional and better-characterized standard materials are still needed. A National Reference System for Clinical Chemistry (NRSCC) will require a hierarchy of materials, ranging from thoroughly-characterized pure forms of analytes to biological samples which contain either or both exogenous and endogenous sources of the analytes.

Before materials and their use in a NRSCC can be discussed, a consistent system of terminology must be agreed upon in order to avoid confusion, misinterpretation, and misunderstanding. Clinical chemistry does not currently have such a system, with its terminology for analytical materials varying both nationally and internationally. As Mitchell has indicated (22), "The present limited nomenclature in current use in the area of reference technology has evolved by trial and error and by cooperation between research workers and field testers." For example, a thoroughly characterized material certified as having specific chemical and physical properties is designated by the National Bureau of Standards (NBS) as a Standard Reference Material (SRM), whereas in Europe the same material is called Certified Reference Material (CRM). Consequently, efforts are being made both nationally and internationally to develop a consistent nomenclature. The International Federation for Clinical Chemistry (IFCC) has published a set of definitions pertinent to reference technology (29), and other organizations are working on similar lists. .

Until this consistent nomenclature is available, reference terminology must be defined in the context of a specific situation. In this paper, we have designated

materials used in a unified reference system as "standard" or "reference" materials. The terms "standard" and "reference" have been applied so widely in overlapping areas that operational definitions are necessary to differentiate them. We define standard material as a single lot of material that is recognized as the final arbiter of chemical composition, chemical reactivity, and mass (or reactive quantity) for a given analyte. We define reference material as any material used in evaluating the accuracy of an analytical method or the performance of other materials used to establish or verify quantitative accuracy. Strictly speaking, a standard material could also be used as a reference material.

An additional distinction must be made between standards and calibrating materials. The application of the term "standard material" in a recent IFCC recommendation to any material used in calibrating an analytical system is quite inconsistent with the above definition and, in our view, is a step backward. Only one standard material for a given analyte should be recognized by a unified national measurement system at any given time. Although similar preparations may be indistinguishable from the standard, their validity can only be confirmed against the recognized standard. Therefore, these unique standard materials must be developed and certified by an official process that is universally recognized as providing the unique material basis of quantitative accuracy and qualitative composition. We submit that nomenclature should be functional in that, e.g., the term "standard" should refer to a material which serves as a unique reference point, and that "calibration material" or some other such term be applied to material used to calibrate a quantitative measurement. (A standard material can be used as a calibration or reference material.)

Another nebulous pair of terms is "primary" vs. "secondary" materials. Although the term "primary standard material" has never been clearly defined, its status as the ultimate reference material for a chemically defined compound has been generally implied. These terms must be clarified if they are to be retained for use in clinical chemistry. We suggest the following definitions for consideration:

(1) "Primary standard material." Any material that is

accepted or officially recognized as the unique standard material in a NRSCC, regardless of its level of purity or analyte content.

(2) "Secondary material." Any material with the same properties but of different origin or lot, even if it is of comparable quality, would be by our definition a "secondary" material and would have to be validated against the appropriate primary standard material. Because a universal supply of any standard material will be limited, high-quality "secondary materials" must be readily and economically available to supply the vast needs of clinical laboratories and of industry. This need is dramatized by the decreasing supply of several international "reference standards" and the efforts of WHO to promote the preparation and maintenance of secondary materials as "national standards."

In this paper, we consider only standard and reference materials that will be recognized and certified as components of the NRSCC. The essential characteristics and requirements that pertain to these materials can later be used as models in deriving acceptability criteria for equivalent secondary materials.

2. Standard Materials

As stated previously, we define a standard material as one that is universally recognized as the final arbiter of chemical composition, chemical reactivity, and mass (or reactive quantity) for a given analyte. Other materials currently used in calibrating or evaluating instruments and other measurement systems are commonly referred to as "standards," but this discussion is limited to analyte standard materials.

Given that a standard material must be clearly identified as the sole, documented lot of homogeneous material against which other materials and analytical measurements are to be validated, it should be the "best currently available" material that defines chemical composition, purity, and mass. Thus, depending on the analyte, standard materials may range rather widely in purity and chemical characterization.

Classification

Several plans have been proposed for classifying various quality levels of standard materials according to their analyte content or degree of purity. We differentiate standard materials as follows:

"Chemically Definable" Standard Materials:

This group comprises those materials of known purity or content whose chemical and physical properties have been thoroughly characterized. Although this group comprises those materials which are generally regarded as high purity materials (e.g., sodium chloride, glucose, cholesterol, and elemental iron), their measured purity may change with advancements in technology.

"Interim" Standard Materials:

This group comprises those materials for which there is currently no independent or exactly replicable basis for assigning a mass content to the principal component or analyte of interest, but for which an analytical need is fulfilled by assigning numerical values to the analyte. A number of national or international "reference standards" for as-yet-uncharacterized protein hormones and certain immunoreactive proteins fall into this category. Because these ordinarily are analytes which are difficult or impossible to define chemically and thus are of unknown molecular composition they would be classed as reference materials unless adopted by broad consensus as interim quantitative standards.

The essential distinction of this group is that this quantitative basis is recognized as being a consensus value, applicable only to one specific lot of material for which exact replication is probably impossible. Assigned values may be in entirely arbitrary units, often defined as equivalents to some mass unit of material, or obtained by consensus analysis in terms of current method-defined analytical units. In order to minimize lot-to-lot variability and possible alterations in the quantitative basis of analyte content, the preparation protocol must be strictly adhered to and the assignment of a concentration

value on a new lot must be based on that of the previous lot.

Specific Applications

The intended application(s) of any standard material must be specified and its qualifications and limitations for fulfilling these purposes must be thoroughly documented. The following represent major applications:

The Reference Point for Physical and Chemical Properties of a Compound or Analyte.

The physical properties and the chemical reactivity of a chemical entity can only be accurately measured against a standard composed of the same type of material in the purest form available. Most of these measurable characteristics are assigned numerical values that can be used as criteria of purity and content for other lots of material such as analytical calibrators for routine use and isotopically labelled compounds. The standard materials can thus be used to arbitrate numerous analytical considerations such as the specificity of applied chemical or enzymatic reactions and separation or isolation reactions and manipulations.

The Reference Point for Quantitative Calibration.

Similarly, the magnitude of response of an analyte in applying chemical reactions or physical properties to quantitative analysis can be defined only with the analyte material itself. This provides the key "dimension" in any analytical result--the reactive mass or molecular equivalent of the substance. Chemically defined standard materials can provide this calibration base as either the quantity of standard weighed into an analysis mixture or as a standard solution in which the concentration is defined by preparation (see Section 3.1).

Preparation of High-Quality Reference Materials.

Although solutions of a standard material in defined matrices can be used as reference materials for evaluating analytical accuracy, sample-simulating matrices such as serum are usually superior for testing the complete analytical

system. Standard materials weighed into matrices in which the analyte is definitely absent can be extremely valuable for these purposes.

As Standards of Quality for Critical Reagents.

Another use for recognized standard materials is as high-purity analytical reagents in certain analytical systems (e.g., trace elemental and enzymatic analyses). In this application the standard material would generally be regarded as the material with which all other candidate analytical reagents are compared.

Essential Characteristics and Requirements.

The following characteristics are essential in any recognized standard material. Although the degree to which these "requirements" can be met varies by substance, each standard should represent the highest quality possible.

Purity (Quality).

"Purity" is a relative term which relates both to chemical and physical characterization. Estimates of purity are usually based on data obtained from direct assays in which fundamental quantities such as mass, time, length, or electric current are measured. In some cases, the level of impurity must be determined, because for some materials the estimate of purity can only be expressed as the degree of impurity. Estimates of purity obviously depend on the state of the art of current measurement technology, and assessments of purity will change as technology improves.

Thorough physical characterization of chemically defined standard materials is an essential criterion of purity. Typical data which must be provided on distinctive physical characteristics of the compound include pH, solubility, density, melting point, optical activity, wavelengths of absorbance maxima, and molar absorptivity. In addition, the specific conditions under which these physical constants were determined must be described. Although some of the physical data such as melting point, density, and molar absorptivity may also be used in estimating the purity of a material, some users require the physical data as well as a description of

the estimates of impurity.

As much information about the nature of accompanying components of the diluent matrices of interim standard materials is needed as for those of chemically definable standard materials. Many analytes for which it is useful to establish interim materials as standards are often difficult to purify, are unstable, or their physical and chemical properties are changed upon purification. Many of these materials are merely concentrates of natural materials. With their complex and undefined matrices, these materials must have their possible matrix contributions delineated if they are to be used as analytical standards. When the material is a mixture of biologically or chemically reactive analyte forms in serum, its use as a calibrator must be limited to specific, detailed procedures in order to avoid problems of method selectivity and nonspecificity.

For some analytes, separating and identifying isomeric forms is vital because different isomers may exhibit significantly different chemical and physical properties. Also, the physical and chemical characteristics of the standard material must represent those of the analyte for which it is the standard. For example, the standard should be in the same isomeric form as the predominant in vivo form. If the same isomer cannot be produced because it is unstable or difficult to manufacture, evidence must be presented that the isomeric forms have the same essential properties and reactivity as the predominant in vivo forms. If the latter is not possible, the material should be classified as an interim standard material

Content (Quantity).

A standard material's major function is to serve as the primary reference point for calibrating all quantitative analyses in which the analytical response of a test sample is directly compared with that of a known quantity of the analyte. In practice, this comparison is made with secondary calibration materials that must ultimately be traceable to the standard material. Content (concentration or mole fraction of substance) must be assigned on the basis of a combination of analytical approaches:

Independent measures of substance that do not depend upon the material being calibrated with the analyte. Such measures include careful estimates of elemental composition, content assays of such physical parameters as coulometric equivalents and gravimetry, and potentiometric estimates of reactive properties. Estimates of mole purity by such techniques as differential scanning calorimetry against other existing standards and estimates of the sum of analyzed values for known impurities.

Regardless of approach, such estimates are of two types:

Estimates of minimum content:

The purer a material is, the less discriminating are estimates of content for small differences, and they may only confirm some value for minimum content, e.g., "99.6% or better." Modern techniques that permit reliable measurements in the "three nines" concentration range have minimized the need to use minimum values for many substances, but for a great many purified biochemical analytes it may only be feasible to represent concentration as lying between some minimum value and 100%.

Best estimates of a mean value:

In current manufacturing practice, one analytical procedure considered to be the most appropriate for the particular analyte in a given matrix is used to determine its mean content value. Obviously, a number of such quantitative estimates may be made for a standard material and should be presented in the documentation. This poses a problem of assigning a "best value," which must be resolved for each analyte standard.

For a standard material of undefined chemical composition whose content is assigned on a consensus basis, quantity is established on the basis of the standard lot of material itself. The value assigned to this material may be selected as representing arbitrary "units" per milliliter or per milligram of substance, or it may be determined by some method-defined response such as absorbance. However, such assayed values are usually obtained by consensus.

In addition to its assigned value, a statement of the method(s) by which the value was derived and an estimate of confidence in the value should accompany the standard material.

Homogeneity

A high degree of homogeneity must be achieved in preparing any certified lot of standard material, and it must be maintained so throughout the packaging process. This homogeneity must be confirmed by statistically valid sampling and testing procedures. Homogeneity should be tested during the manufacturing process to assure complete mixing of the product and again after the product is packaged since composition can be altered by (e.g.) flame sealing of ampoules, adsorption and leaching phenomena with the container surfaces, and the quality and quantity of an inert packaging gas (30). Direct and indirect methods of determining product content and composition should be used to detect any heterogeneity in the final product.

Stability

Although stability is often used as an absolute term (i.e., a material is either stable or unstable), it is actually relative to the time over which any material will exhibit some physical or chemical change of state or composition. The terms "stable" and "stability" should denote the absence of any evidence of chemical or physical change over a designated time period.

Types of physical changes include: (a) changes in physical state, e.g., solid to a gas; (b) structural changes, e.g., changes in lattice structure or isomerization--a particular problem in clinical chemistry because of the increasing use of analyses involving an enzymatic substrate analyses; (c) radioactive decay, which can be handled as a predictable rate of change (in contrast with most other physical changes). Although it is not usually a stability problem, the isotopic ratio of different sources of a given element can affect the formula weight of the product; lithium salts are a prime example.

Chemical instability

Materials may be altered by chemical processes such as oxidation, reduction, or hydrolysis. Since these processes are usually catalyzed by traces of such impurities as metals, oxygen, and moisture, these impurities must be carefully excluded from the material while it is manufactured and packaged.

Microbiological instability

Microbiological contamination is not usually a significant problem with most chemically defined, suitably packaged standard materials, but the physical and chemical characteristics of some materials can be significantly altered by microbiological growth if appropriate steps are not taken to prevent it. If a material can be affected by microbiological contamination, it must be produced under sterile conditions.

Assessment of stability

Before a substance or product can be considered a suitable standard material, its stability over a stated time period must be demonstrated. A material is usually considered stable if no changes in its chemical or physical properties can be found after it is subjected to such rigors as elevated temperatures, light, freezing and thawing, and mechanical shaking. Ideally, a standard material should be stable over a wide temperature range and should not require unusual storage temperature conditions. The material should be unaffected by storage for long periods in the range 0°C - 50°C. Its stability should be monitored during each phase of the manufacturing, dispensing, and packaging processes so that the source of any instability can be pinpointed. The stability of the material must be reaffirmed after its container is opened and before it is used. Stability testing is very demanding and requires a thoroughly validated statistical model that takes into account such factors as the number of containers in the batch, the degree of confidence required, precision of the analytical technique, and the sampling plan.

Certification and Documentation

A necessary requirement for any standard material in the NRSCC is that it be clearly recognized as the only current standard with which other materials are compared and all analytical methods are calibrated. This recognition entails a procedure whereby the material is declared and documented to be a national standard by a recognized certification body. This procedure should be conducted under a national consensus mechanism incorporating steps and criteria established prior to the introduction of a new standard:

The certifying body must be officially delegated to establish material standards by a clearly defined process which is available for evaluation by all concerned groups.

Certification cannot be merely the end process or final stamp of approval without risking a heterogeneous collection of nonconforming standards. The process of developing the standard must therefore be integrally related to the final certification.

A candidate standard material must meet certain criteria before it can be considered for certification. Both general and specific criteria need to be developed for individual analytes.

The scientifically acceptable methodologic basis must be established for quantifying analyte and impurities, homogeneity, and any other measurements deemed necessary.

The permissible applications and constraints on use of the material must be specified.

Availability

Although any certified lot of standard material is more or less limited in quantity and may be difficult to reproduce, it must be readily available in order to fulfil its function. Because there may be a need in many cases to limit not only indiscriminate consumption but also the universal availability of a standard material, there must be some means of determining and providing ready accessibility for legitimate needs of the NRSCC. The use of certified

standards of all types can also be anticipated to increase, particularly because they will be used as daily calibrators in reference analyses for regulatory purposes. Much consumption of this type could probably be reduced by using materials which can be substituted for standard materials without detectable loss in the accuracy of the calibration. Demand for materials that are easy to reproduce be met by frequent replacement. However, with other materials it may be necessary to establish a parallel system of primary secondary standards in which the secondary replacement lots are certified as being "identical" within given confidence limits with the parent primary material.

In any case, rather large quantities of standard materials must be prepared for such activities as research and development, characterizing and validating secondary standards and calibration materials, and validating conformity between national and international accuracy bases. Thus, each certified lot should be stable for at least several years under specified conditions.

Replicability

Although any standard material should eventually be improved upon and be replaced to meet new demands, certifying any lot indicates that it can be successfully replicated at the same quality level for as long as it is needed. This assurance can only be provided by documentation that the product actually has been successfully replicated. Although steps may increase the cost and effort in developing a standard, greater costs and confusion have previously resulted from the failure to reproduce acceptable standards.

Many interim materials derived from biological sources can be expected to be difficult to replicate exactly, primarily because of variability in the source materials. In order to minimize lot-to-lot variability and possible inconsistencies in the basis for quantitating analyte content, the preparation protocol must be adhered to and the assigned concentration value of a new lot of an interim standard material must be compared with that of the previous lot.

Production and Distribution

Preparation. The manufacturing protocol for preparing standard materials must be strictly adhered to and thoroughly documented. All pertinent information regarding the manufacture of such a material (such as temperature logs, raw materials employed, and in-process quality control data) must be recorded and maintained.

Testing and Validation

Before a standard material is manufactured, raw materials must be thoroughly tested before they are used in the process. During its manufacture, a material should be tested at regular intervals in order to monitor the reaction or purification process and the homogeneity of the product. Testing should be performed on the bulk, finished product and also on the material after it is packaged. It should be retested when taken from its container and before it is used. After material testing has been completed, the validation data should be available for scientific review before the product is officially recognized as a standard material.

Dispensing and Packaging

Care must be taken during the dispensing process to ensure that the integrity of the material is maintained. The product must be continuously mixed during dispensing, the material handled under aseptic conditions (if needed), and any other precautions taken to assure product quality. The amount or volume of product dispensed must be accurately controlled, especially when the material is to be lyophilized.

Since the material may be stored in its container for several years, the environment to which the product is exposed is of critical importance. The quality and quantity of packaging gases may be of particular importance in maintaining the quality of the product. Only containers consisting of materials in which there is no exchange of the material, water, or gas with the atmosphere should be used. Other factors such as light stability of the product, cost, and size, shape, and tightness of the seal should also be considered in determining the most suitable container.

Container surfaces should be inert and should not exhibit adsorption or desorption (leaching) effects. Containers should be cleaned and sterilized before they are used in order to avoid chemical, biological, or particulate contamination. Cleaning solutions or solvents which impart a "memory effect" (e.g., dichromate) should not be used.

Labelling

All in vitro diagnostic products must comply with the labelling requirements given in the *Federal Register*, Vol. 28, No. 50, Pages 7096-7102, March 15, 1973.

Storage and Handling

Ideally, a standard material should not exhibit undesirable handling characteristics such as hygroscopicity, a tendency to adsorb carbon dioxide, temperature instability, or instability in the presence of light or air. If a material does exhibit any of these characteristics after the container is opened, handling instructions included with the product should so instruct the consumer. The protocol should include recommended techniques for weighing, dissolving, and storing solutions prepared from the standard as well as stability information for the material remaining in a previously opened container and suggestions for removing inherent or incurred impurities such as occluded moisture or absorbed carbon dioxide.

If the material is a potential health hazard to those handling it, any information regarding the danger and the recommended handling procedure should be clearly stated both in the protocol and on the container.

Distribution

The facility or organization which has the responsibility for distributing an officially recognized standard material will probably also inherit such other custodial functions as periodic retesting, inventorying, and properly maintaining materials. A computerized data management system would facilitate these and other functions such as accurately signalling the need to manufacture more of a standard material. Since amounts of officially recognized

standard materials will be limited, the latter mechanism will be an important aspect of properly utilizing such products.

3. Reference Materials

Here the term "reference material" is applied to any material used in evaluating the reliability and performance of an analytical method in which some sample-simulating material is subsequently used to establish or verify accuracy. When such a reference material is a recognized compound of NRSCC, its defined level of quality must be validated. The requirements and characteristics discussed here are intended to apply only to these NRSCC materials, with a mechanism for such validation being assumed.

Classification

Reference materials can be categorized on the basis of the level of quality of the standard analyte material available, the nature and quality of the matrix in which the analyte exists or is introduced, and the intended use of the reference material. However, there are two major categories:

Materials whose quantity or concentration of analyte (i.e., known quantity in terms of mass or volume) is established by the preparation of a standard material dispensed into a known final mass or volume with a diluent known to be free of the analyte. The analyte concentration is a function of the purity and composition of the standard material and of the accuracy of the gravimetric and volumetric manipulations involved in preparing it.

Materials of native or added analyte, in any matrix for which the desired concentration is achieved by quantitative analysis. These materials might range from standard materials dissolved in pure solvents to spiked i.e., analyte-supplemented) or unspiked serum. Further subclassification (e.g., for reference materials prepared by weighing specific quantities of a standard material into a carefully analyzed matrix with a normal or low analyte background) might be useful.

Uses

NRSCC reference materials are used primarily in interrelating and assessing the analytical performance and reliability of the various methodological components of the National Reference System.

Methodology Development

Reference materials are useful in the development of an accuracy base of both definitive and reference methods.

Definitive Method

During the development and validation of a definitive method, reference materials will be useful in establishing and assessing the accuracy base of the definitive method and in transferring this base to other studies.

Reference Method

The major uses for reference materials in developing and maintaining reference methods are:

(a) In evaluating the accuracy of a candidate reference method being developed and in comparing it with a definitive method.

(b) In testing the interlaboratory transferability of a consensus reference method.

(c) In assessing and/or assuring the stability of methodology over time. For maximum effectiveness, analyses must be performed with verified reference and definitive methods and made available on a regularly scheduled or requested basis.

Transfer of Established Accuracy Bases

These uses include:

Testing the performance of diagnostic products.

Assessing laboratory performance; the primary function

here would be in relating the accuracy of reference values in laboratory certification to the NRSCC.

Validating the transfer of NRSCC quantitative values to in-house benchmark materials in industry, research, and clinical laboratories.

Matrix Materials

A reference material is composed of the analyte and its matrix, with the latter being defined as the environment surrounding a given analyte. The type of synthetic or natural material used as a matrix will be chosen on the basis of the product's intended use and may vary from a chemically definable solvent-solute system to a complex, biological matrix such as serum.

Synthetic Materials

Synthetic matrices have been used in an attempt to minimize or control uncertainties that often result from the effects of natural components in an undefined biological material such as pooled serum. In addition, synthetic materials are usually more widely available and would be expected to have less lot-to-lot and batch-to-batch variation than do natural materials. However, the ultimate utility of a reference material that is produced in a synthetic matrix will depend, not on these practical advantages, but on its ability to simulate a sample as it is normally analyzed.

Materials used as synthetic matrices include those from biological (e.g., bovine or human serum albumin) and nonbiological (e.g., polyvinylpyrrolidone and polyethyleneglycol) sources. In documenting a synthetic matrix, the nature, source, and amount of and the purpose for including all components must be given, and specimens must be subjected to the same testing for stability and homogeneity as natural materials.

Native Sample Materials

To ensure that the chemical and physical properties of reference materials simulate those of native samples, the former are often prepared in a natural serum matrix.

Although sera from either human or animal donors have been used, the trend has been to use human sera. However, because of the diversity in diet patterns and health status of the donor population and the increasing difficulty of obtaining sufficient quantities of human materials, using pooled human serum as the matrix may not always be preferable.

When pooled human serum is used to prepare reference materials, the following characteristics of the donor population should be defined, described, and, if possible, controlled: (a) age and sex distribution, (b) dietary status, (c) medications taken, and (d) whether health status has been verified as normal or characteristic of a particular disease. Additional information should include whether each individual specimen was aseptically collected, handled, and tested; whether other treatments such as use of antibiotics were used to avoid contamination; or whether bacterially contaminated material was submitted to sterile filtration. In the latter instance, breakdown products or substances emitted from lysed microorganisms may not be removed by filtering. Attempts to control denaturation caused by heat, light, and other conditions should be described, as should any procedures used to remove denatured components.

Although the physical and chemical characteristics of animal fluids and tissues are usually more definable than those from humans, the question of whether or not the animal specimens can simulate those from humans must be carefully considered. Animal tissues may contain analytes normally absent in human materials, or amounts and forms of common components may differ. Differences in protein components are likely to be pronounced between animal and human materials. Different amino acid sequences may affect both separation techniques and quantitation procedures. Although animal matrices may be preferable for many analytes, for certain analytes such as enzymes the effects of intra-species differences must be assessed in interpreting results.

When a natural matrix is to be used in preparing a reference material, the feasibility of chemically or physically removing from it any of the relevant analytes should be considered. This process results in a "zero-base" or analyte-free pool. Reference materials containing specified quantities of the given analyte can then be

prepared by spiking the matrix with known quantities of a standard material.

Additives

Various chemical components such as preservatives or stabilizers may be added to the natural matrix. Such a component should be definable, should not result in methodological bias, and its purity, amount, and source must be described.

Materials Preparation

A product-formulation protocol must be rigidly adhered to when preparing a reference material. Initially, a specified quantity of an analyte* is added to a bulk quantity of the specified matrix. The resulting bulk material is dispensed into individual containers after which the pool is evaluated, stored, and distributed as necessary. A mandatory criterion for any reference material is that the contents of individual containers all be identical within acceptable limits of concentration, activity, potency, homogeneity, and stability.

Preferably, matrix material used in preparing a lot of reference material should be obtained from a single source and lot number. If it is necessary to pool a number of batches or lots of matrix material, homogeneity of the resulting material must be demonstrated and analytically evaluated regardless of studies conducted on the individual batches. Experienced personnel should handle and process the material. After any analyte is added, the bulk material should be demonstrated to have sufficient stability and specific activity to serve its intended purpose. (Biological materials of very high purity are often unstable, so that

*Although it may be desirable to combine multiple analytes in a single preparation for analyte groups such as electrolytes, including multiple, unrelated analytes in one reference material may require large quantitites of material for stability testing and analysis, and may result in other logistical problems. For such procedures as methodologic comparisions, usually one analyte is involved.

less pure materials may be preferable if they are more stable.)

Preparation of Pooled Bulk Material

A bulk quantity of the reference material is often prepared by adding the desired quantity of the analyte as a standard material to a specific quantity of matrix. Measurement and transfer of the quantitative components may be either gravimetric or volumetric according to predetermined performance specifications. After all components are added, the pool is thoroughly mixed to obtain a homogeneous bulk mixture. In a preparation of high quality, homogeneity cannot ordinarily be confirmed by analysis to the degree of significance required by a national reference material, thus homogeneity must be assured by the mixing process. In order to ensure that packaged material is homogeneous, all units should originate from the same bulk preparation and should be prepared in a single working session.

Container Requirements

The container for the final packaged unit must fulfill two essential funtions:

Provide containment:

The container must be impenetrable to external contamination and to the components of the contained material (including water) and in most cases atmospheric gases. It must be resistant to breakage, it must be securely sealed, and must be capable of withstanding the sterilization process and normal handling.

Be unreactive:

Neither the container nor closure must contribute nor remove chemical or physical components to or from the sample.

Dispensing Into Individual Containers

Regardless of the final form of the product, bulk

materials are preferably dispensed as liquids because they are easily handled and because superior precision can be obtained with widely available current liquid dispensing equipment. In order to assure that homogeneity is achieved and maintained during the dispensing process, it may be necessary to keep the bulk material at a constant temperature and to stir it constantly.

In some cases, the bulk material may be in a solid form. If so, it may be preferable to dissolve or suspend it in an appropriate solvent for the reasons stated above. Obviously, the solvent selected must have no deleterious effect on the product or on the assay system in which it will be used. When the solids are relatively insoluble or unstable in a liquid form, the bulk material should be dispensed as a powder. Unless each lot of material is to be resampled by weight, the dispensed quantity must be accurately and precisely delivered to assure a specified final concentration of reconstituted liquid. In all cases, the ultimate homogeneity of the product must be insured.

Liquid versus Lyophilized Samples

Dispensed specimens are normally stored in lyophilized or liquid form, both of which have advantages and disadvantages.

Liquid Samples - Although liquid specimens are not subject to problems of volumetric reconstitution, they must be frozen for storage and possibly for shipment. Also, liquid samples are more likely to interact with the container, and evaporation must be avoided. In addition, secure closure must be assured, and storage conditions must be carefully controlled.

Lyophilized Samples - Processing biological reference materials by lyophilization offers several advantages, including better stability and increased shelf-life; however, some analytes (notably glucose) require additional measures to ensure stability. The stability of lyophilized specimens is critically dependent upon a very low content of residual moisture. Moisture control must be strictly maintained during preparation, both in terms of volume dispensed and homogeneity. Details for reconstitution, including pipetting

techniques, must be specified, and the reconstituting solvent must either be supplied or specifications given for preparing it. Since lyophilization may alter chemical or physical forms of many of the components, lyophilized materials must be tested both before and after they are prepared in order to determine whether reaction characteristics have been affected.

CDC recommends that liquid products are preferred to lyophilized ones if the necessary stability can be demonstrated.

Characterization

In order to ensure that a reference material is reliable, homogeneity and stability testing must be performed after it is prepared, preferably after it is stored in its container. (Some materials are stored under inert gases and the quality and quantity of gas in vials may be variable.) Flame-sealing ampoules may affect the stability of the material, and some liquid samples may require equilibration with the surface of the container before analysis.

Homogeneity Testing

Homogeneity testing should be performed with a statistically valid model to rule out vial-to-vial material variability. The model chosen depends on several factors such as the number of vials in a given lot, the precision of the analytical method employed, the degree of confidence required, and how frequently the material is manufactured. Although homogeneity testing must be performed on the final product, it is also used throughout the preparation to monitor and verify the quality of the material.

Stability Testing

Stability, like homogeneity, must be monitored throughout the preparation. In addition, it must be constantly monitored during storage and confirmed under various extremes of shipping conditions. The effect of temperature and the shelf-life of the product can be projected from its reaction to storage at different times and temperatures, and proper storage conditions for the product can be ascertained.

Stability and proper storage conditions should be determined for the product after its container is opened and the contents of the individual vials are reconstituted or thawed. Other factors of stability such as light sensitivity, physical denaturation, reconstitution, and the effects of freezing-thawing should also be tested.

Assignment of Target Values

Three mechanisms can be used for assigning target values for reference materials.

Analysis

Reference method values are established and assigned to reference material by use of reference methodology and an accepted statistically-designed protocol.

In addition to the assigned values, documentation for a reference material should include analytical values obtained with various analytical techniques and methods. Such information documents matrix effects that are likely to occur with these techniques and methods. They should not be considered as certified values.

Quantitative Addition

Quantitative addition is usually used only for analytes that are normally absent in the biological sample or in which a "zero-base" pool has been artifically created either from synthetic mixtures or by procedures for removing the analyte from a natural material. In such situations an exact quantity of a standard material or the analyte is diluted to a specified quantity or volume with the "zero-base" pool. Values are then calculated and assigned to the materials.

Quantitative Addition and Analysis

When addition and analysis are combined, an analyzed base pool is prepared at low concentration, an exact quantity of the analyte is added to form an elevated pool, and various portions of the resulting two pools are quantitatively combined to produce pools with intermediate concentrations of the analyte. Although the analyte concentration of the low

pool is analytically determined, precisely establishing added analyte and dilution ratios permits calculation of the theoretical concentration intervals of the analyte in each of the intermediate pools. Comparison of the theoretical with the measured values can be used for confirmation.

Requirements for validation by each of the above approaches or combinations of approaches must be outlined by this Conference, and a system for certification of reference material must be established. In addition, recommendations must be made for setting acceptable uncertainty intervals, i.e., whether they will be based upon the normal range, medical usefulness, the state of the measurement and preparative arts, or some other criteria.

Documentation

It is mandatory that complete and detailed written documentation be provided for a reference material. This documentation will serve as a written "pedigree" for the material and should cover the time from the introduction of the material throughout the period of use until the material is depleted or replaced. The documentation should include (a) recommendations of consulting groups, (b) descriptions of all components, (c) descriptions of the preparation and dispensing processes, (d) an exact protocol for reconstituting and using lyophilized material and for thawing and using frozen materials, (e) results of homogeneity and stability testing, (f) data used for setting target values and uncertainty limits, (g) conditions of storage and shipment, and (h) handling precautions, such as for any potential biological hazard.

Availability, Distribution, and Custodianship

A reference material should be readily available and obtainable within a reasonably short period of time. Consequently, because reference materials are expected to be widely used in an NRSCC, their manufacture, characterization, validation, and storage will be an expensive, time-consuming and continuing task.

V. METHODS

1. Introduction

Developing, implementing, and maintaining a National Reference System for Clinical Chemistry (NRSCC) based on a definitive accuracy base involves several essential components, including both definitive and reference methodology. It is recognized that reference methods are not yet available for all of the analytes of clinical interest and that a concentrated effort will be required to develop them. Consequently, we recommend that provisional reference principles should be formulated for those analytes for which acceptable methodology can be determined relatively rapidly by consensus to be used on an interim basis. This interim solution could be approached either by: (a) selecting a published method as an interim reference, or (b) establishing an analytical principle (or combination of principles) which would permit some flexibility of choice of the exact procedure. In the following sections, the need for and development of reference technology for the three types of methods will be discussed.

2. Analytical Models

Clinical laboratories must assay for hundreds of different analytes, ranging from the simplest of elements to the most complex of organic molecules, which are often present in trace quantities in a complex and variable matrix into which a variety of drugs and/or their metabolites might have been added. With this complex analytical situation, we recommend that a priority list of analytes be generated to allow a series of reference methods to be developed in a logical and systematic manner. This priority list will be influenced by both medical and analytical considerations and should be the product of a collaborative and coordinated effort of representatives from (1) the professional societies that represent the clinical laboratory, (2) industry, and (3) government.

After an analyte has been assigned a priority, it must have been or must be chemically and physically defined. Unfortunately, either many of these analytes have not been isolated and purified in a sufficient quantity for chemical

or physical characterization or a satisfactory methodological base has not been developed for measurement. Thus, it is unrealistic to expect one measurement system to provide a traceable accuracy base for all analytes, necessitating the development of a family of analytical models into which various analytes can be appropriately placed. Examples of such models include:

Analytes Having a Defined Accuracy Base

Analytes in this category can be chemically and physically defined so that a satisfactory measurement system will be available. Examples include electrolytes, trace metals, glucose, low-molecular-weight nitrogenous compounds, and steroids such as cholesterol and cortisol.

Analytes Having an Assigned Accuracy Base

Analytes in this category are those which can be satisfactorily analyzed with existing methods, but which lack a definable accuracy base (e.g., a standard material or a definitive method) or which require a relative accuracy base defined by test parameters. To ensure reproducibility of measurements for these analytes, rigorous standardization and specification of the chemical and instrumental parameters used to perform the assay measurements will be necessary. Examples of this group include enzymes, the peptide and protein hormones, immunoglobulins and other specific proteins, glycoproteins, lipoproteins, and glycolipids. Also included are analytes assayed by techniques in which complex biological molecules are used as reagents (e.g., radioimmunoassay, with an antibody as a reagent).

Analytes Having a Semidefined Accuracy Base

This category includes (1) analytes which have only some of the components necessary for a validated measurement system and (2) analytes of research interest which have not yet been adequately characterized and tested. For analytes in the first group, a standard material may be available but a definitive method may be lacking, or vice versa.

Nonphysiological Analytes

This category contains analytes not normally present in physiological fluids. They may have the essential components for measurement, but the appropriate method may be complex and difficult to standardize and document. In such situations, emphasis will be placed on the preparation of matrixed reference materials made from standard addition of the analyte to zero-based serum. Transfer of reference technology will be via these reference materials.

3. Uses of Reference Methodology

A National Reference System will require that a hierarchy of methods be developed, each having different and, in some cases, similar uses.

Definitive Method

A definitive method in the NRSCC is used primarily to establish an analytical value (derived from a base system of measurement) which is accepted as the most accurate estimate of the absolute concentration of an analyte in a given fluid. When a definitive method has been exhaustively evaluated and demonstrated to have no known source of inaccuracy or ambiguity, it will serve as the accuracy base for long-term characterization and validation of reference methods.

Reference Method

A reference method in the NRSCC is used primarily in transferring an accuracy base to the participating clinical laboratories. In this transfer, a reference method may be used in:

Monitoring accuracy by comparing results being checked with those obtained by a technique whose accuracy has been documented.

Demonstrating validity and stability of an analytical process or reference material.

Evaluating and validating new or modified reference methods.

Assessing field methods and commercial diagnostic products.

Analyzing matrixed reference material for internal and external quality control programs. The reference method will be used to certify the analytical values assigned to the reference material.

Assisting in developing and subsequently evaluating and validating newly developed analytical instrumentation.

Monitoring (in conjunction with definitive methods) the effects of drugs or their metabolites upon analytical results obtained with field or routine methods.

Providing evaluation scales for government regulation of laboratory performance of products used in diagnostic testing.

Provisional Reference Principles

Provisional reference principles serve many of the functions of reference methods. Other specific uses of provisional reference principles and materials include:

Use in voluntary laboratory standardization for analytes for which no definitive and reference methods are available. Users of such standardization programs will logically be (a) manufacturers of diagnostic products who need an accepted reference point for instrument/method/material development to meet legal requirements, (b) regional reference laboratories providing services to other laboratories, and (c) university, commercial, and government laboratories engaged in medical research requiring common reference points for epidemiologic validity.

Use in establishing multianalyte reference networks.

Unstructured uses such as individual laboratories and manufacturers which set up and maintain provisional in-house reference principles.

Provisional reference principles which will be selected on the basis of past reliability, will obviously be candidates for reference methods when existing methodology is evaluated.

4. Characteristics and Requirements of Reference Methodology

The methods comprising the hierarchy of the NRSCC will have individual characteristics and requirements.

Scientific Validity

All reference methodology must be based on sound scientific principles.

Definability

Definitive Method. A definitive method will be the accuracy base for a given analyte. Definitive methods will be developed and validated in an institution(s) which has the prerequisite technological expertise. A definitive method must be theoretically sound, documented, and published so that the clinical laboratory community can evaluate it.

Reference Method

The documentation of a reference method must be sufficient to define clearly all of the critical parameters required to replicate the conditions for a stable, reproducible analytical procedure. The primary assumption for a reference method is that it can be exactly reproduced in time and place solely on the basis of its procedural specifications. A second level of definition is also required that details a means for achieving standardized deliveries of volume and mass, reproducible conditions such as temperature and pH, and the materials and reagents used in the analysis. The method must have built-in validity checks to demonstrate that all critical parameters have been reproduced within required tolerances.

Provisional Reference Principles

Definability of provisional reference principles is limited by the degree of official consensus reached on the specific requirements for determining a given analyte. Documentation may only prescribe in general terms the reaction system used and minimum instrument and reagent requirements, with reference to one or more well-accepted

published procedures which describe the analytical principle. Conversely, an existing method may be so clearly superior (yet lack the requirements and documentation of a reference or definitive method) that it may be specified in considerable detail. Obviously, flexibility must be maintained in specifying the degree of definition warranted by the status of a given method, and definitions should be reviewed, perhaps annually, to confirm that utility and relationship to the state of the art for a given assay are maintained.

Accuracy and Specificity

Definitive Method

A definitive method should provide the most accurate estimate possible of the "true value" of a given analyte. Thus, it is considered to have no known source of inaccuracies or ambiguities. In the strictest sense, a definitive method will be specific for a given analyte.

Reference Method

A reference method is one of proven accuracy which is primarily used to measure bias and specificity. Ideally, it has minimal error and bias, and its analytical relationship to the definitive accuracy base will have been extensively investigated, validated, and documented.

Provisional Reference Principles

Accuracy and specificity of provisional reference principles cannot be clearly defined because they do not relate to an official accuracy base. However, information on specificity will be available as candidate reference methods are validated. Comparing candidate reference methods to official provisional reference principles should be an integral part of this process. Provisional reference principles provide a "provisional accuracy basis," with recognized and hidden limitations that must be taken into account by the user.

Precision

Definitive Method

The imprecision associated with a definitive method should be minimal since it will (1) decrease the number of replicate analyses required to statistically establish confidence in any measured values and (2) increase the statistical discrimination offered by the definitive method.

Reference Method

For the reasons discussed above, attaining a specified degree of precision is usually a prerequisite for any reference method (14).

Provisional Reference Principles

Since "provisional" implies temporary, all costs involved with achieving a given level of statistical confidence for a "provisional target value" must be kept at a minimum.

Performance Specifications

Definitive Method

Performance specifications will be required for each definitive method. Details of such specifications will vary among methods, depending on the analytical goals set for each. Performance criteria must be specified for the accuracy and precision expected for each method and for the confidence limits into which the measured results should fall.

Reference Method

Detailed performance specifications will be required for each reference method. Details of such specifications will vary from method to method depending on the analytical goals set for each analyte. Performance criteria shall be specified for the accuracy and precision expected for each method and for the confidence limits into which the measured results should fall. Also, methodological and instrumental performance specifications are required for the individual

steps or operations in the method. For example, performance specifications should be stated for gravimetric and volumetric measurements (such as preparing standards, samples, and reagents) required of a method and for other instrumental variables such as spectrophotometric performance. Although pragmatic consideration may be given in setting these limits, in no case should they lead to or result in decreased analytical performance.

Provisional Reference Principles

Performance specifications for provisional reference principles will vary considerably from analyte to analyte depending upon the state of the art of the procedures which are selected by consensus. These requirements may be limited to basic analytical principles such as optimum wavelength for measurement, preferred temperature, general reagent specifications, and method of calibration, but will not be as detailed as those for a definitive or reference method unless the policy setting group deems necessary.

Reliability

Definitive Method

A definitive method must have long-term reliability to avoid an uncertain or shifting accuracy base. Since a definitive method will usually be developed, implemented, and maintained within a single institution, emphasis will be placed on intralaboratory reliability.

Reference Method

A reference method must be capable of exact interlaboratory and intralaboratory replicability and maintenance over an extended period of time. It must be sufficiently reliable to withstand inevitable small changes in its chemical and physical environment without analytical results being affected. Any parameter drifting must be adjustable and a reference method must include a means of recognizing and controlling random and systematic performance-induced error.

Provisional Reference Principles

Reliability is as important for a provisional reference principle as for a reference method. However, since all details of the former may not be as fully specified as they would be in a reference method, a somewhat lower level of reliability may be expected.

Availability

Definitive Method

A definitive method must be used to check the performance of its associated reference method periodically and to evaluate and validate a proposed candidate reference method. Because a definitive method is an important and expensive analytical tool which should not be used indiscriminately, a set of guidelines must be developed on when it should be used.

Reference Method

A primary characteristic of a reference method is that it must be sufficiently widely available to fulfill its function as an operational basic point or reference. Consequently, it must be transferable to multiple laboratories, the number and types of which will vary depending upon the performance specifications that are an integral component of every reference method.

Provisional Reference Principles

The availability of provisional reference principles is synonymous with transferability at the level of definition required for provisional principles since provisional principles, to be effective, must be available for use by many laboratories.

Transferability

Definitive Method

Since a definitive method is developed, implemented, and maintained at a highly technical and eminently qualified

laboratory, its transferability is not mandatory. However, Brown (18) has raised the question of the desirability of validating the results of a definitive method by transferring the method and testing it elsewhere. If consensus dictates that this validation is needed, transferability becomes an important requirement, and detailed documentation will be required to allow transfer.

Reference Method

A reference method must be exactly replicable over time in a number of laboratories. The extent to which a reference method can be successfully transferred and maintained among appropriate laboratories will depend upon the degree of reliability and precision demanded for a given analyte, on the availability of materials, equipment, personnel, and on the application of detailed statistical protocols to evaluate the transfer and to maintain the method once it has been successfully transferred.

Transferring and maintaining a reference method will be time consuming and will require a certain degree of support, which laboratories must be willing to provide. However, reference methods should be designed and developed for the widest possible use within the constraints necessary to replicate the performance of the method as it was originally validated. It is anticipated that the number of laboratories using a given reference method will be relatively small.

Provisional Reference Principles

Since provisional reference principles must be used by many laboratories to be effective, they should be accompanied by documentation and descriptions of chemicals, instruments, and conditions. Transferability of provisional reference principles will be greatly enhanced by the availability of flexible and voluntary clinical chemistry standardization programs. Such programs may be based upon provisional reference materials, characterized in central reference laboratories which use the provisional reference principles.

Productivity

Definitive Method

The time required per analysis is less important for a definitive method since emphasis is placed on the analytical quality of the results and not on practical considerations such as analysis rate; however, practical considerations cannot be completely ignored. Therefore, a definitive method must be capable of producing results within a time frame which is compatible with the needs of the NRSCC. In addition, if the imprecision of the definitive method is relatively high, time becomes a more important consideration, because replicate analyses must be done to validate results.

Reference Method

A reference method will have multiple uses, which will generate both low- and high-volume work loads. Consequently, a reference method should provide such alternatives as a manual procedure for low-volume application and a mechanized or automated procedure for high-volume application. However, unique mechanization or automation, in which essential analytical parameter(s) cannot be defined and independently replicated and validated, cannot be employed. The method must allow for efficient and economic use of resources with no sacrifice in quality. All alternative approaches must be validated.

Provisional Reference Principles

Productivity and cost effectiveness of provisional reference principles relate directly to the precision requirements discussed previously. Because they are categorized as "provisional" a large expenditure of manpower, money, and time may not be justifiable.

Costs

Definitive Method

Developing, implementing, and maintaining a definitive method will be an expensive process requiring analytical expertise, sophisticated instrumentation, and resources.

Consequently, cost per se is not a primary factor in determining cost-effectiveness.

Reference Method

Considerations of cost/benefit are definitely important for a reference method. Productivity must be considered when the overall method is designed and when any component or step is selected and specified. Balancing cost vs. benefit requires recognizing essential requirements, setting performance specifications for each procedural step, and selecting the mix of components that can achieve these targets most efficiently. No candidate reference method can be rejected on the basis of economic considerations alone, unless the costs are so high that they limit the method's availability, transferability, and productivity below the levels required by a reference method.

Provisional Reference Principles

Cost effectiveness of provisional reference principles relates directly to their precision requirements. Because they are categorized as "provisional," they may not warrant large expense.

5. Development of Reference Methodology

The NRSCC will depend on the availability of a hierarchy of analytical methods. Methods must be developed, implemented, and maintained for each of the various classifications.

Definitive Method

Several factors must be considered in developing a definitive method. They include:

Selection of Analytical Principles

A definitive method must be based on proven and accepted scientific principles which emanate from a sound theoretical and fundamental foundation. Meeting this requirement will entail having available technical centers of excellence, composed of an extremely competent scientific staff supported

with a sophisticated technological base. The few institutions fulfilling this description will have the responsibility of selecting the most appropriate analytical principle from which the definitive method is developed. However, the rationale used in the selection should be disseminated and published in order to achieve scientific review and consensus approval and acceptance. This documentation should include detailed discussions of the theoretical basis of the proposed principles and of the protocol for the analytical process.

In general, only a few techniques meet the criteria for a definitive method. They include isotope dilution mass spectrometry (IDMS), calorimetry, and other techniques which involve the measurement of such fundamental properties as temperature, pressure, and time. To date, IDMS has been almost exclusively utilized for the development of definitive method of clinical interest and have been limited to inorganic and organic analytes with relatively low molecular weights. However, with technological advancements the molecular weight limitation will not apply. The increased availability of ultra high resolution mass spectrometers has greatly expanded the range of analytes which can be analyzed with IDMS-based definitive methods.

Unit operations in the Development of an IDMS Definitive Method

IDMS has long been recognized as a very accurate and sensitive method for analyzing inorganic analytes in various matrices. The technique is basically a form of assay by internal standard, in which the standard is an isotopically definable form of the analyte and the quantitative ratio measurement (internal standard/analyte) is an isotope ratio. Thus a calibrated amount of artificially enriched isotope or labelled analyte is added to the sample, and the isotopic composition of the mixture determined. If the isotopic composition of the "normal" analyte is known, the amount present in the original sample can be derived from the following relationship:

$$x = y(A_n/A_s)(S_k/N_k)\left(\frac{S_{ik} - M_{ik}}{M_{ik} - N_{ik}}\right)$$

Where:

x = mass of the normal analyte.
y = mass of the spiked or labelled analyte.
A_n = atomic or molecular mass of the normal analyte.
A_s = atomic or molecular mass of the spiked or labelled analyte.
N_k = atomic or molecular abundance of the K^{th} isotope or K^{th} isotopic configuration of the normal analyte.
S_k = atomic or molecular abundance of the K^{th} isotope or K^{th} isotopic configuration of the spiked or labelled analyte.
N_{ik} = ratio of the i^{th} and K^{th} isotope or isotopic configuration in the normal analyte.
S_{ik} = ratio of the i^{th} and K^{th} isotope or isotopic configuration in the spiked or labelled analyte.
M_{ik} = ratio of the i^{th} and K^{th} isotopes or isotopic configuration in the measured mixture.

The high degree of accuracy obtainable with IDMS techniques results from the following:

All chemical manipulations are done on a weight basis involving straightforward stoichiometric separation and reaction. These manipulations can often be performed without introducing systematic errors into the analysis, in which case the only known error consists of experimental random error.

Only ratio mass spectrometric measurements are involved in the quantitation step; absolute determination of the quantity of a given isotope present in the sample is not necessary. By requiring only the measurement of isotopic ratios the requirements of instrumentation calibration and/or correction are drastically reduced.

In order to generate acceptable definitive values, each of the following analytical steps must be carefully performed.

Procurement and validation of the labelled analyte

The chief requirement for using IDMS in developing a definitive method is the availability of a characterized quantity of an isotopically-stable* form of the analyte. For most inorganic analytes, several stable isotopes are analytes, several stable isotopes are available. However, for organic analytes, the availability of the labelled analyte is often the most critical factor in developing a definitive method. An additional problem in the procurement process is the need to demonstrate and validate that the physical and chemical characteristics of the unlabelled and labelled analyte are identical. It is implicit in an IDMS method that no physical or chemical discrimination exists between the unlabelled and labelled forms of the analyte during any step of the analytical process.

Thus, it can be anticipated that the successful development of a definitive method for a given analyte depends on the availability of the labelled analyte and the demonstration that no isotope effect occurs between the unlabelled and labelled forms of the analyte. This validation of the absence of isotope effect becomes very difficult, especially if extensive separation, derivatization, and purification steps are required before mass spectrometry. However, to establish the validity of the definitive method, each step in the analytical process must be critically examined. It is likely that procuring and validating the labelled analyte will be an expensive and time-consuming process.

Mass analysis of the labeled analyte

After a labelled analyte has been procured and validated, its atomic abundance (S_k) is determined by mass-spectrometric analysis. In some cases this may require that the sample be pretreated by chemical derivitization, purification, and separation.

*In principle, it might be practical to use a long-lived radioactive isotope as the spike, but it is usually considered unwise to introduce a radioactive material into a general-purpose mass spectrometer system.

Addition of labelled analyte to unlabelled analyte. One of the unique and advantageous characteristics of IDMS in developing definitive methodology is the fact that it does not require absolute measurements. For example, its basic measurement is determining the isotopic ratio value between the unlabelled and labelled analyte. However, one step in the analytical process requires the absolute measurement of a known quantity of labelled analyte (y) which is added to a known quantity of the biological matrix containing the unlabelled analyte. This is usually a gravimetric addition which is quite accurate as it is traceable to a set of NBS certified weights. It should be noted that another critical step in the addition process is the thorough mixing of the unlabelled and labelled forms of the analyte. A successful IDMS analysis requires a homogeneous sample.

Separation of analyte for mass analysis

After the unlabelled and labelled forms of the analyte have been mixed, an aliquot of the homogeneous mixture is processed to obtain a quantity of the pure analyte. Various techniques used include thin-layer and liquid chromatography. In addition, for those organic analytes that are volatile or that can be derivatized to a volatile state, a gas chromatograph coupled directly to the mass spectrometer is a very useful tool for separating and purifying the analyte. Each of the steps of the separation process must be critically studied to ensure the absence of isotope or matrix effects.

Mass spectral analysis

The accuracy of a IDMS method is dependent on the reliability and resolution of the mass spectrometer. Since the basic calculation in an IDMS method involves measuring isotopic ratios, the mass spectrometer must provide reproducible results within the time frame that the sample and labelled standard are analyzed. As the time between these analyses becomes longer, the requirement for rigorous standardization and control of the critical instrumental parameters becomes more necessary. Problems which might arise in the mass spectral analysis include (a) fractionation of inorganic isotopes, (b) presence of commonly occurring stable isotopes, and (c) fragmentation patterns.

Minimizing matrix effects

Definitive methods must be designed with which to analyze a given analyte in a specified biological matrix. Ideally, definitive methods should be free of systematic errors resulting from sample matrix effects. However, as the chemical structure of organic analytes becomes more complex, the matrix effects and variability of the sample tend to become more difficult to control. This problem is especially acute in the analysis of complex analytes such as drugs and/or their metabolites.

Sample matrix effects for most inorganic analytes can be effectively reduced or removed by destroying the biologic matrix by wet or thermal ashing.

Reagent and physical facility specifications

Trace contamination from inadequately cleaned reagents, equipment, glassware, and/or inadequate control of environmental conditions in the laboratory can represent a major source of imprecision or bias. Laboratories incapable of producing ultra-clean reagents and glassware and which do not have access to clean working areas of Class 100 or better should not attempt to develop definitive methods. Adequate facilities for synthesizing and purifying isotopically labelled standard material are essential for developing such methodology.

Validation

Validation includes establishing all performance criteria of the method, especially precision, freedom from interferences, and ability to meet the challenge of "difficult" specimens.

Validating the accuracy of a definitive method is a very different procedure from those used for reference and routine methods. The validity of the accuracy base of a definitive method is dependent on the scientific wisdom exhibited in selecting the basic analytical principles or techniques to be used and the direct traceability of all absolute measurements to such "certified" standards as NBS certified weights.

Validating the precision of a definitive method consists of analyzing a number of well-defined or certified samples and then statistically evaluating the resulting data to gauge whether the present analytical goals have been achieved.

After the validation is completed*, the initiating laboratory prepares a detailed written report and widely disseminates it to the scientific community in order to obtain scientific review and consensus acceptance. For pragmatic reasons, a panel of qualified experts could be selected and convened to evaluate the results prior to widespread dissemination.

Documentation

Since a definitive method is apt to be developed, validated, and maintained within a single institution, it can be argued that the documentation of the method needs only to be provided in sufficient detail to satisfy the requirements of that institution. However, a thorough documentation would be advantageous for several reasons:

The documentation should be widely disseminated to the scientific clinical community to achieve scientific review and consensus acceptance, thereby obviating the stigma attached to the lack of interlaboratory validation of a definitive method.

Because the methodology would be used only periodically, detailed documentation would assist in maintaining the definitive method at the initiating institution.

If transfer of the definitive method became necessary, detailed documentation would be necessary. This is true both for intralaboratory transfer necessitated by personnel changes and for interlaboratory transfer of the definitive methodology.

*Note: Part of the validation of the definitive method may be conducted in parallel with the development and validation of a reference method.

Transfer and Maintenance

Transferring the definitive method to another institution will only be considered if the consensus of the concerned parties indicates the need for it. Maintaining the definitive method is necessary since it will be used to check the performance of the reference method periodically and to validate new reference methodologies as they are developed. This will place a considerable burden on the initiating laboratory and will require the availability and use of carefully prepared and stored certified materials and further depends upon complete and total documentation of operational aspects of the methods.

Reference Method

A successful reference method is developed systematically. The steps include: Evaluation of Existing Methodology (Including Technology).

Several methods are available for measuring most analytes. In addition, new techniques have resulted from technological developments and recent advancements in the rapidly expanding fields of electronics and data processing. For a given analyte, each of the existing methods must be identified and preliminarily evaluated as to its suitability as a candidate reference method.

The initial evaluation of the existing methodology involves a group of qualified experts who survey the literature and objectively select the most promising candidate method(s) whose attributes and characteristics most nearly meet the scientific, analytical, and practical criteria required by a reference method. This panel will be composed of representatives of the diagnostic industry, professional societies, and government.

When a group of methods is considered as potential candidate reference methods, in some cases the attributes of a particular method may be so clearly superior that it can be selected as the candidate reference method and consequently would be so developed. In most cases, two or three methods may qualify, and evaluated further in order to select a candidate reference method. This multimethod evaluation

study may be conducted by a single laboratory or by a group of laboratories, each having expertise with one or more of the candidate methods. However, in a multilaboratory study, coordination becomes a practical problem and in such a situation we recommend that one laboratory initiate and coordinate the study.

Optimization

Once a candidate reference method has been selected, the essential parameters that affect its analytical performance are identified and optimized. The basic assumption underlying optimization is that the best combination of stability, sensitivity, reproducibility, and specificity will be achieved by optimizing the analytical conditions of the measurement process. Conditions of the measurement process are sought which maximize the analyte response and minimize the response of the measurement process to known or suspected interferences and to random noise.

Validation

After the optimization studies are completed and the results documented and reviewed by the panel of qualified experts, the first version of a written, methodological protocol is prepared for the candidate reference method. Subsequently, an internal validation study is designed and conducted to evaluate this initial protocol. This internal validation study is usually assigned to the laboratory that developed the candidate reference method and is based on strict adherence to the method protocol.

During the validation study, the accuracy base of the candidate reference method is rigorously evaluated by different approaches, depending on the specific analyte, the availability of a definitive method, and a standard material. These approaches include:

Analyzing a common set of samples with both the definitive and the candidate reference method.

Analyzing a set of samples containing known quantities of the standard material as a supplement to both defined and undefined matrices.

Analyzing a common set of samples with the candidate reference method and alternate methodology based on completely different analytical principles.

All of these procedures are followed by comparison and statistical evaluation of results.

When neither a definitive method nor a standard material is available, the validity of the method is established by demonstrating and documenting that:

The analytical process is such that substances other than the analyte usually encountered in the specimen population do not bias the analytical results.

The analytical process has been studied in enough depth to establish optimal analytical conditions with respect to reagent concentrations and equipment and instrumental stability.

The calibration process is based on an appropriate reference material, whose preparation is predictable, reproducible, well-characterized, and/or on physical properties independently measured.

An integral step in establishing the accuracy base for a candidate reference method is testing its specificity, by challenging it with samples containing compounds that are known or suspected to interfere. Interfering compounds are endogenous (i.e., the physiological constituents typically found in body fluids and those compounds related primarily to diet or drugs [31]) or exogenous (i.e., contaminants from blood collection and processing techniques or commonly used reagents and their contaminants). Thus, there are hundreds of compounds that are potential interferences. Fortunately, it is possible in most cases, with the knowledge available about the reaction and procedures involved in the measurement process, to predict potential interferences. Thus, a pragmatic approach must be taken in designing and implementing a protocol for interference studies (23,32) and in selecting compounds and sample sources to be used in such studies. Endogenous compounds which should be tested include those that typically would be expected to interfere and those that are typically present in the greatest concentration in

body fluids. Because numerous drugs are now being administered (31), a test protocol (32) must be developed and implemented. Testing must be conducted on samples from various pharmacological categories (e.g., analgesics, anticonvulsants, and antihistamines) and also on samples from patients taking known medication. Although the latter will be difficult to obtain, it is important that they be tested. It is obvious that any observed interference should be thoroughly evaluated and the results included in the documentation of the method.

After the validation study is completed, the results are documented, statistically evaluated, and discussed with the expert panel. Necessary revisions are made in the protocol, which is then submitted for an independent validation to assure (1) that it conforms with the general guidelines (See Section II) in content and format and (2) its accuracy, precision, specificity and workability as a procedure. A concise report is prepared which is then submitted for review to determine whether the method is ready for transfer. At this point, any major objections and questions should be discussed, considered and answered.

Transferability Testing

After the validation studies are complete and the method has received national consensus, the method is then tested for its ability to be transferred to other laboratories.* This process is conducted under the guidance of a project team that monitors and evaluates the test.

The project team designs the protocol of the transferability test, provides the testing materials, and submits the method to a group of several selected laboratories. The laboratories chosen to participate in the study must all have competent personnel and be properly

*Practical experience gained by transferring a reference method has indicated the wisdom of conducting an in-house transfer experiment with personnel that have not been associated in the development of the reference method. Many problems can be quickly identified and corrected before beginning the more extensive and expensive transfer testing.

equipped and willing to perform the exacting work designated by the protocol.

For efficiency and economy, a central laboratory is chosen and delegated the responsibility of coordinating and conducting the study. Initially, the central laboratory prepares and/or collects the necessary specimens and distributes the protocol, instructions, and specimens to the cooperating laboratories. In actual practice, this step in the transferability test is conducted sequentially as a series of round robin studies:

Round Robin I: The protocol, specimens, and all reagents (each purchased from a single lot) are provided to each of the participating laboratories by the central laboratory. Efficacy and stability of the reagents are determined by the central laboratory.

Round Robin II: The protocol, specimens, and key reagents are provided by the central laboratory, and the participating laboratories purchase and prepare the other reagents.

Round Robin III: Only the protocol and the specimens are provided by the central laboratory. The participating laboratories purchase all reagents used in the study and test them for efficacy before using.

Each round robin study is conducted according to a specific time schedule, after which the analytical data and other pertinent information are returned to the central laboratory for collation and summarization. At the end of each round robin study, personnel of the central and participating laboratories and the expert panel evaluate the data to determine whether the analytical performance of the candidate reference method statistically meets the previously set accuracy goal. If the analytical performance of the method is acceptable, the transferability testing then moves to the next round robin study. If, however, the analytical goals have not been met, personnel of the central and participating laboratories and the expert panel try to identify and pinpoint sources of error and procedural problems or ambiguities in the method. The method protocol is revised accordingly and the round robin study repeated

until the analytical goals have been met or until it is evident that the goals were set too high.

After the three round robin studies have been successfully completed, a fourth is conducted in which a common set of samples is supplied to all of the participating laboratories and to the national and/or international laboratory which developed the definitive method. The samples are analyzed and the results returned to the central laboratory for data processing. The results obtained from the candidate reference and definitive methods are statistically compared in order to validate the accuracy of the candidate reference method. Any bias must be thoroughly studied and documented. For analytes for which there is no definitive method, other approaches to demonstrate accuracy must be taken.

Upon completion of the validation and transfer studies, a comprehensive report is prepared by the central laboratory and submitted to the participating laboratories and the expert panel for editorial review and comments. (After it is formally accepted, copies of this document are provided to all interested parties for comment and ultimate consensus acceptance.) This document contains the final reference method protocol, which is also reported in the scientific literature or in special issuances by professional societies or government.

After the transfer of the reference method has been demonstrated successfully by the selected laboratories, it must then be transferred to all of the laboratories that will use it. Several means are available for effecting this transfer:

Expanded transfer studies conducted by professional societies.

Publication of the method in scientific journals and other acceptable publications.

Clinical chemistry standardization programs based upon the reference method.

Manufacturers' assistance or standardization programs.

Maintenance

Once transferability has been confirmed and the method accepted by the consensus process, it must be maintained and used regularly. A viable NRSCC requires the availability of both definitive and reference analyses on a regularly scheduled and/or a request basis. Maintaining reference methods and transferring them requires the long-term availability of stable, well-characterized reference materials. Because such materials are expensive to produce and provide, designated government facilities may be delegated the responsibility. Subsequently, at frequent intervals, these programs should be reviewed by panels comprised of representatives from the various professional organizations, industry, and government.

Documentation

A reference method must be carefully documented as it must be reproducible and repeatable in both time and place on the basis of procedural specifications and detail alone. Such documentation should be written in an objective, clear, and concise manner. Documentation will include:

Instrumental and equipment performance specifications

Documentation of the reference method includes general performance specifications for the primary instrumentation and other ancillary equipment required. In addition, the documentation should include instructions or references for assessing the performance of such components. The actual values that are listed for the various performance specifications will depend on the analyte and the method. However, tolerance limits should be set as narrowly as is economically feasible, placing more stringent requirements on the instrumental performance specifications than may be warranted or justified on the basis of the analytical principles involved. This limitation removes a degree of uncertainty from the method and may increase the specificity of the method by reducing or eliminating the effect of various interferences.

Chemical specifications and availability

The purity and source of every reagent required in the method must be specified and documented. ACS specifications for reagent purity usually will suffice, but if they do not, the quality and purity of the reagent required will be specified. The requirements for reagent purity should be reasonable to avoid increased costs and decreased availability and stability of the reagent. Reagents required by a reference method should be available from several different sources.

Preparation of reagents

Detailed information and instructions will be provided for preparing individual and combined reagents. Documentation will include procedures for validating the analytical quality and efficacy of reagents, and information concerning the stability of reagents under specified conditions of time and temperature.

Specimen collection and storage

Documentation will include information concerning the techniques used to collect, process, and store the specimens. Factors that are considered include:

(a) type of specimen (e.g., serum vs. plasma)
(b) stability of analyte
(c) time requirements
(d) bacterial and chemical contamination
(e) temperature restrictions
(f) sealing of storage containers
(g) solubility problems and/or potential deterioration of the specimen from repeated thawing, evaporation, and contamination
(h) anticoagulants and preservatives

Sample and standard preparation and requirements

Detailed instructions for preparing samples and standards for analysis will be included in the documentation. The effects of time and temperature on the stability of samples and standards should be given.

Analytical procedure

Successful transfer of the method depends primarily on the ability of analysts working in different labs to follow procedural specifications and reproduce analytical performance. Consequently, thorough and detailed documentation of the analytical procedure is one of the most critical operations in its development. The analytical procedure must be documented on a step-by-step basis and should be described clearly and concisely in a standardized format.

Interferences

Documentation of the reference method should include qualitative and quantitative information on compounds that interfere with the method.

Data Processing Procedure

The exact procedure for data manipulation including rules for including and excluding data.

Appendix

This section includes background information concerning the development of the reference method and how the reference method compares analytically with other methods. Because such documentation is extensive, this section may be available only upon request from a selected facility.

Provisional Reference Principles

The criteria for selecting a set of provisional reference principles for a given analyte are similar to those for a reference or definitive method, but the emphasis is more on the pragmatic aspects of a "provisional" or temporary reference point and is very dependent upon the process of making a selection based upon consensus.

A recognized panel of experts from private and government sectors will select and coordinate provisional reference principles and will compile the information base

required to develop uiform documents that describe provisional principles.

ACHIEVING CONSENSUS AGREEMENT

The process of achieving consensus agreement may be described in six stages:

Stage 1. The accumulation of recommendations (solicited from users and other interested parties) by the expert panel.

Stage 2. Complete and thorough review of the existing literature with critical evaluation by the panel.

Stage 3. Issuance of a preliminary recommendation by the expert panel for a given method principle with reference to an existing published method or methods. This issuance would be supported by (1) the critical conclusions derived from a review of published existing methods; (2) summary and conclusions derived from the recommendations of individual experts, manufacturers, and professional societies; and (3) the comments of expert consultants also involved in the review process.

Stage 4. A period to allow for comment on the recommendations from the profession.

Stage 5. Final publication of a "Provisional Reference Principle."

Stage 6. The mechanism for gathering comments after publication should be efficient and guarantee that a meaningful exchange of information takes place. This information will be especially useful in selecting a candidate reference method, especially when it is very similar to the provisional principle. Ideally, the process leading to the selection and publication of a provisional principle should be thorough enough to keep "complaints" and updating of decisions to a minimum.

Intra- and Interlaboratory Validation

Selection of provisional reference principles is based on a pragmatic approach and is coordinated by the expert

panel. However, to ensure that a valid principle has been selected, it should undergo a validation process. This process will be limited to principles required for validating definitive and reference methods. The approach used to validate a reference method can be modified for the validation of a provisional reference principle.

Documentation

Documentation must be adequate to describe the set of analytical and chemical principles necessary to establish the procedure in multiple-user laboratories with a fair degree of reliability and reproducibility. Obviously, the detail with which a set of principles is described will depend upon the extent of consensus agreement among selecting experts with regard to those analytical and methodological factors considered critical. Many requirements of a set of provisional principles can be expected to evolve over time as more information about a given method is accumulated during the evaluative stages leading to the development and validation of reference and definitive methods. Although the basics of a set of provisional principles should not change if an interim reference point is to be maintained, allowances should be made in documentation for including new information which (1) more clearly defines the principles; (2) improves quality of analytical results; and (3) makes the method more precise, efficient, and ensures a constant relative accuracy (or bias). Specific areas requiring detailed documentation are:

Instrument and equipment performance specifications should clearly set acceptable limits on instrument performance for those steps which are important to the outcome of analytical results. The width of these limits will be method dependent and should not be established arbitrarily. Examples include (a) a repetitively used sampling pipet which should maintain high precision but may be relatively inaccurate when samples and standards are treated exactly alike during pipetting; or (b) spectrophotometric bandpass which may be on the order of 10-15 nm if a method is known to produce a broad chromogen peak at that wavelength with no known interferences, thus allowing the use of a filter spectrophotometer.

Chemical specifications and availability should be documented

in detail to the extent that these factors are known to be important to the satisfactory performance of provisional reference principles. Again, the extent of documentation will be dependent on available knowledge. For example, it may be sufficient to describe an extraction solvent only as "Isopropanol," but if it is known that water and peroxides in certain lots of isopropanol lead to erroneous results, requirements for this solvent must be specified in some detail and, when possible should include techniques for satisfactory purification.

Requirements for preparation of reagents should be specified in sufficient detail to ensure that known critical steps in reagent preparation are performed correctly.

Specimen collection and storage conditions should be specified in sufficient detail to ensure adequate performance.

Wherever critical, stability information and details of samples and standard preparation should be clearly stated.

Analytical procedures should be stated in clear, simple, straightforward language and in adequate detail. It should be kept in mind that provisional principles are intended for use by a large number of laboratory facilities of various levels of sophistication.

Known interferences should be documented.

Transfer and Maintenance

Transfer and maintenance of provisional reference principles must occur if a provisional reference point is to be established for a given analyte.
Publication of a provisional reference principle by the panel of experts in the recognized scientific literature or in special issuances by professional societies or governmental agencies offers the most effective transfer mechanism. Transfer may be further augmented by workshops and training sessions conducted at national meetings of professional societies. Further assistance will be available in formats similar to CDC's Manufacturers Assistance Program and Clinical Chemistry Standardization Programs.

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FOOD AND DRUG ADMINISTRATION

POSITION PAPER

by

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INTRODUCTION

In 1972, the Food and Drug Admininistration (FDA) declared its intention to regulate all *in vitro* diagnostic products. At that time the procedure for implementing such a program was developed in consultation with the Center for Disease Control (CDC), the National Bureau of Standards (NBS), and the FDA Diagnostic Products Advisory Committee. During the initial phase of developing the diagnostic products program, the need for a national clinical laboratory reference system, reference methods and materials was identified. Since that time the FDA has been engaged in activities to establish a sound basis of reference for the clinical laboratory. The FDA provided funding to the National Bureau of Standards over a three-year period for the development of reference methods for twelve (12) analytes for which analyses are commonly performed in the clinical chemistry laboratory. This task was carried out in collaboration with CDC, with input from several other areas of clinical laboratory expertise. This work is expected to be completed by the end of 1977. Additionally, the FDA contracted with NCCLS to develop methodological principles for the assignment of analyte concentration values to assayed calibrators by a consensus mechanism. Methodological principles for eighteen (18) analytes have been developed by this process. The Agency welcomes the opportunity to co-sponsor and participate in this Conference and share its views on issues facing the health care community.

The goal of providing clinical chemistry *in vitro* diagnostic products which employ methodologies with sufficient levels of accuracy, precision, and specificity is viewed as necessary and achievable. Furthermore, it is fully recognized that in order for this goal to become reality, a national, uniform approach is required. The foundation of this approach must be based on the availability of validated reference materials, reference methods, and testing procedures, as well as a common language employing sound definitions. The FDA has long held the position that full representation from all segments of the clinical chemistry community must be involved in the development and implementation of approaches that will assure the availability of reliable clinical laboratory data to the physician.

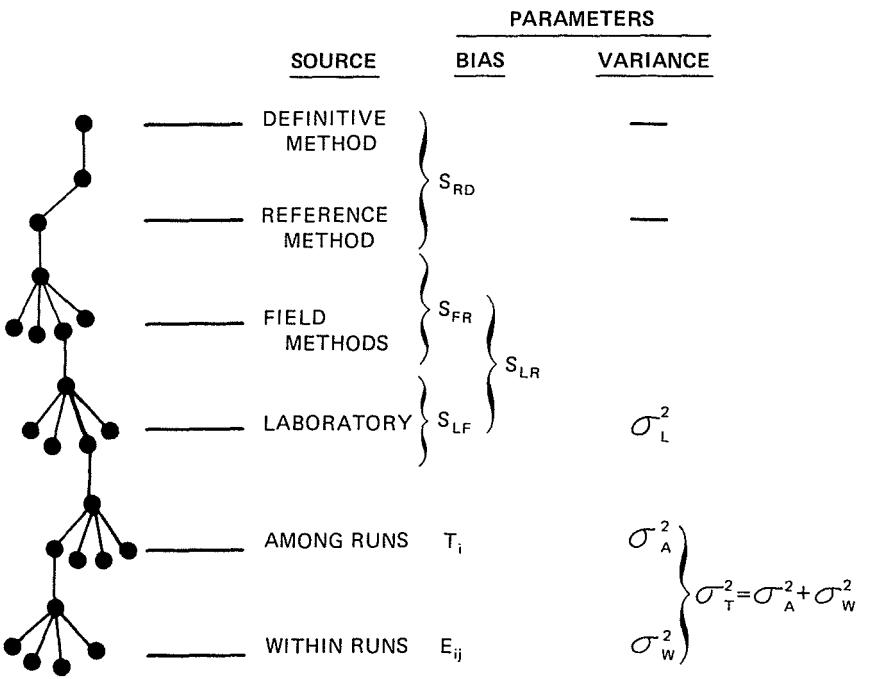

Figure 1. ERROR STRUCTURE OF MEASUREMENT (REFERENCE) SYSTEM

The primary goal of a national clinical measurement system is the improvement of patient care. The sharing of views on common concerns will constitute a major contribution towards that end. It is the author's firm belief that all relevant issues must be clearly identified, then followed by the development of implementation approaches that are both reasonable and scientifically sound. Needless to say, the benefits will be profound.

The many benefits of a sound national reference system are quite obvious. It would provide a platform from which many important FDA decisions could be made.

ORGANIZATIONAL DEVELOPMENT OF THE NATIONAL REFERENCE SYSTEM

Analytical Goals and Quality Requirements

The most fundamental function of the clinical chemistry laboratory is the measurement of specimen analyte concentrations. The result of this measurement process is, strictly speaking, an inference about the "true" (= definitive) concentration of the specimen and may be expressed in many formats, e.g., a point estimate + a standard error or coefficient of variation for the estimate, or perhaps more conveniently, a 95 percent confidence interval. Figure 1 illustrates the error structure of a typical measurement (or reference) system. It contains four types of methods: definitive - whose limiting mean value will be defined as the "true" concentration; reference - of known bias to definitive method, of known interference, adequately small variance components, feasible to implement in reference laboratories; field - various implementations of various methodologies found in routine field laboratories; laboratory - implementation of a field method in a particular laboratory. The parameters of the error structure are: biases - reference to definitive, field to reference, laboratory to field; variance components (random errors) - among laboratories, among runs, within runs. These parameters may vary with concentration. Bias is the difference between limiting mean values; it is the sum of systematic errors.

If estimates of appropriate error-structure parameters are available, a field laboratory result can be "corrected"

for laboratory-to-reference and reference-to-definitive biases. However, the cost of debiasing is the addition of the variances of the bias estimates to the variance of the laboratory result. Because of this, and the fact that ancillary medical data (e.g., normal range or reference and discrimination value data) would suffer the same increased uncertainty in the transformation to definitive method units, there appears to be a decrease in utility by this transformation. There may be a considerable improvement in the utility of an analyte determination if medical reference values are - if not measured by the reference method - transformed to reference method units and promulgated in reference method units. This idea should be thoroughly studied. An obvious benefit is that data from different laboratories or different field methods could be combined or compared.

Clinical chemistry measurements are used in three fundamental medical decision processes: (1) screening, (2) single observations, (3) trend analysis. The screening process (see Figure 2) entails prior knowledge of the normal analyte distribution, the particular disease analyte distribution, the prevalence of the particular disease, as well as the relative costs of false positives, false negatives and the test itself. From this information an (inverse) measure of utility, a cost function, can be derived, and with it various characteristics of the cost effectiveness of the analyte as well as the effect of analytical error (random and systematic) can be observed. One long range goal for improving the effectiveness of clinical diagnosis should be the careful accumulation of data on analytical distributions of healthy and specific disease populations.

If this distribution data were acquired with the reference method in adequately large and thorough studies and could be transformed for reference use by each laboratory or field method, the unreliable practices of using textbook ranges or inadequate laboratory or field method specific normal ranges could be discontinued.

For most analytes the only presently available criterion for analytical goals may be the one recommended by the 1976 Aspen Conference, namely, limit random analytical error to

one-half of biological variation (including within and among individual). Require that $CV_A \leq 1/2 \sqrt{(CV_{WI}^2 + CV_{AI}^2)}$, where CV_A, CV_{WI}, and CV_{AI} are analytical, within individual, and among individual coefficients of variation, respectively.

Single observations are often used to decide if a patient's analyte concentration is above or below some reference value. Cost functions and the effect of random and systematic error can be derived, but again the limiting factors are biological. A reasonable goal seems to be a requirement that the $CV_A \leq 1/2\ CV_{WI}$.

Analyte observations are often made at specified time intervals in order to detect a trend. In this case decreased precision increases the number of observations necessary to detect a trend of specified slope. This third type of use of clinical chemistry data is not affected by a constant bias, but could be considerably influenced by varying interferences. Within-individual variation is once again a limiting component of variance suggesting the requirement that $CV_A \leq 1/2\ CV_{WI}$.

ORGANIZATIONAL COMPONENT

Requirements: an integrated reference system designed to provide a national scientific basis for quantitative measurements in clinical laboratories: necessary components and required functions.

For measurement to be fully meaningful, there must be a scientifically developed accurate measurement base. Such a base for clinical chemistry has begun to be developed through the joint efforts of the sponsoring organizations and must be expanded and strengthened. A scientific measurement base consists of whatever it takes to enable the performance of meaningful measurements: hierarchies of materials and methods, performance criteria, physical and functional performance tests, people who are well motivated and trained and capable, measurement units, means for communication, quality assurance and statistical guidance, and coordination through a single contact point.

Hierarchies of materials include those used in a particular laboratory for quality control and those used for

calibration, those used for comparison of laboratories or methodologies, and those used to determine the acceptability of materials used in diagnostic products or clinical laboratory procedures. For methodology, the necessary accuracy, precision, specificity, sensitivity, and transferability are achieved and monitored through reference materials and methods. Hierarchies of methods range from routine field to provisional reference to reference to definitive. For reference methods, ease of use and simplicity of operation are not primary goals. Yet such methods cannot be too highly specialized because we need transferability among experts, and among qualified clinical laboratories having technical competence. More is needed than standardizing and controlling routine methods for the clinical laboratory because the ultimate goal of the National Reference System (NRS) is that better, more accurate analytical methods be used routinely.

By performance criteria we mean, first, that for each methodology used in clinical chemistry, there must be an understanding originating from the medical community of clinicians and laboratory physicians and scientists, of the medical meanings and performance requirements of that clinical analysis. Paramount always are matters pertaining to minimizing patient risks and these matters do include the accuracy and precision and specificity and meaningfulness of clinical sampling and analysis. Second, for diagnostic devices that are or include instruments, the performance criteria also include both physical and application figures of merit. An in vitro diagnostic device may be thought of as an entity or an assembly used for the determination of an analyte in a clinical laboratory, for a medical purpose. The methodology, or measurement system, includes the device and all other elements used for the determination of an analyte. It must be possible for the user to find out whether methodology results are trustworthy.

There is still a need for international agreement on the meanings and uses of some of the most basic terms, e.g., accuracy. Accuracy does involve a comparison with a reference. However, in practice, experts select the best current approach to obtain a reference value. As human beings, we can only resort to reference methods that are arbitrarily classified by experts as provisional, or as

reference, or as definitive methods and to materials that are also developed and classified on the basis of recommended uses.

With the enactment of the Medical Device Amendments of 1976, and with pending refinement to the Clinical Laboratory Improvement Act, there is a substantial increase in the responsibilities being given to federal agencies for the oversight and the regulation of the quality of medical devices used in clinical chemistry and of clinical laboratory performance. This mandate requires rational bases for defining and measuring and setting limits to the performance of methodologies and laboratories. For some analytes, there is not yet an adequate scientific measurement base.

We need to expand the base, especially to provide for accuracy limits. The 1976 Aspen Conference on Analytical Goals in Clinical Chemistry distinguished three possible medical testing situations: (1) group screening (2) individual single point testing, and (3) individual multipoint testing (trend analysis). For each situation, Aspen identified different relative demands on performance characteristics. In addition, abundant examples were presented to show that physicians do need accurate results. Also, as performance standards set in terms of needs for patient care, and with concern for cost-effectiveness, the Conference set linearity, range, and precision goals for many analytes and recommended that goals be set for accuracy. Accuracy is important since it is assumed that the numerical value of the test results in a biochemical definition of disease.

We need National Reference System (NRS) reference materials and methods that are acceptable to and used by clinical researchers, manufacturers, and clinical laboratories. Acceptance should be based on meeting agreed upon specific performance specifications and validation and transferability tests. NRS materials and methods must be developed according to priorities and be updated as required and as enabled by experience.

Medically accurate clinical laboratory results are required; i.e. the difference (from all causes) between the amount reported and that present should be medically insignificant in the sense that such differences will not

affect the treatment of the patient. Methodologies must be designed and be used so that for a given sample, all measurers in all locations would provide the same input to the physician's diagnostic process. Accurate results are the only way to have a common language, to describe the analyte present (as opposed to found) in human specimens, independent of who did the test and with what methodology. There must be accurate methodologies for investigative studies, to learn how to sample, how accurate routine results need to be, to learn which numerical values define disease, or what the biologic variability is. The interpretability of clinical research results obtained by various investigators is severely limited unless results are in the same language, and results cannot be directly applied for diagnosis unless the clinical laboratory uses that same language.

The exact trend for an analyte can only be measured with accurate data or if the accuracy does not change. Data must not include unknown and variable and medically significant difference from the amount present. With assured accuracy, even data from various methodologies can be used. Long-term trends for analytes for a patient can only be observed through accurate measurement.

Means

An organizational plan is needed to meet the above requirements by effective and efficient use of available national resources, both government and private, commerical and professional.

Premises

FDA agrees with the stated premises of this Conference. In addition, FDA recognizes that in human affairs there cannot be coordination without a coordinator. Human activities involving members of diverse groups cannot be carried out effectively without central coordination to keep the activities focused and on schedule.

Communication

Excellent communication is an absolute requirement to apportion national resources in effective and efficient ways,

aimed directly at meeting the needs of clinical chemistry for a National Reference System (NRS). One of the essential functions of the NRS is the design and activation of a communication network that functions among the members and beneficiaries of the System. Budget realities will inevitably have their part in molding the administrative details of the communication network, but we must assure that all of those who will contribute and benefit are included. International reference system affairs and affiliations must be continued, fostered, strengthened, and coordinated.

Procedures for Validating Materials and Methods

There must be procedures for validating materials and methods. How can acceptance of a material or a method for specified uses be reached? Where the material or method is placed in the NRS hierarchy and which material or method is chosen for a particular use are not absolute. The label or classification, rather, indicates either current consensus or majority expert opinion regarding how much is known about the characteristics of the material or method, regarding what are the valid uses and needs, and regarding what is the status relative to other materials or methods. When further knowledge is created, the classification or the identity of a member in a hierarchy must be reviewed and may be changed. Another aspect of classification is the major problem that arises when we do not have a whole hierarchy. For example, in the absence of a definitive method, we may have only an NRS provisional reference method.

To say that we must reach a consensus is to be simplistic: to oversimplify by ignoring complexities or complications. At a given point in time, for some real problems, it may be wrong to assume that only a consensus should be reached. Consensus is general agreement, collective judgment, opinion based on reasoning and evaluation by people chosen to reach and state the consensus. The judgments are based on special knowledge and given by experts. Consensus is arbitrary but authoritative, the "best available now" judgment or decision as to what constitutes compatible relationships. For real problems, there may be cases where there can be agreement either because of lack of discord among those present and speaking or because of acceptance of ideas and thus tolerable accommodation. There

may be other issues where there is a consensus regarding most details together with informed dissent concerning other details. In some cases, this may, at the time, be the correct result of the cooperative effort. Where there are items of consensus and items of dissent, there should be published a statement of the problem, a description of reasons and principles for decisions that are reached with consensus, the consensus itself, and the dissenting judgments, the reasons and principles for these. Even when a consensus is reached, the reasons and principles for acceptance and rejection should be provided along with a description of the problems that were addressed. These rational responses to problems, that is, providing the judgments, both consensus and dissenting, and the reasons for acceptance and rejection, should minimize adverse effects of the necessary dependence upon the good sense and judgment of the arbitrarily limited group of experts involved. They will also constitute a rational foundation for appropriate application of the information and recommendations.

In any case, there must be periodic review and updating of consensus and of dissent and of labels and of choices.

Priorities

Since there are many important problems but only finite resources of money, people, and time, priority setting is vital. What are the principles, the criteria for risk, for cost, for alternatives, for benefit? Who sets the priorities? Through the NRS, priority setting must be coordinated for the best interests of the patient. For specific materials and methods, priorities will have to be set in accordance with the particular details involved. Priorities should not be set only on the basis of the percentage of all tests that a test ranks in the nation's clinical laboratories. Benefit and risk to the patient must remain the number one consideration in priority setting. There may be cases where a newer, not-yet-fully-established test calls for an element of an accurate measurement base that should be high on the priority list. As other examples, high priority should be given to providing accurate measurement base elements to the development and proving of biological reference materials and to the development of reference materials or methods where there are only

provisional materials or methods.

Coordination

That there cannot be cogent coordination of the NRS without a coordinator has been stated as a premise. There must be a coordinator who has sufficient uninterrupted time as well as motivation. Motivated voluntary participation needs the support of dedicated full-time planning and direction. There should be one full-time central point of contact for important human endeavors.

The sole full-time task of the NRS could be to identify and accept and recognize needs and to get things done by assignment to and coordination among members, where expertise resides. To get things done, some funding and some authority may be needed. Also, the assembling of groups of experts to advise regarding specific technical, including statistical, tasks can be effectively and efficiently centrally directed in conjunction with the ultimate users of the task results. Moreover, the design and conduct of surveys can be so directed.

One traditional mode of action is to proceed by means of administered and funded agreements and contracts. Considerable accomplishments have been made already by interagency agreements or understandings between FDA and CDC and between FDA and NBS and between CDC and NBS.

There must be central input from medical professionals, including clinicians and including laboratory physicians and scientists. There must also be input from regulators and from measurement experts. Also, there must be one set of specific goals, agreed to, and updated through experience, and followed by all. For the common good, the coordinator will sometimes need to declare by fiat that a workable majority opinion (i.e., items of consensus and some items of dissent), or a consensus (on all items), as the case may be, has been reached. The coordinator must also coordinate outwardly, to other governments and to international standards organizations. He must decree by fiat, but with the agreement of a specific member for whom a task is intended, when specific projects are completed and the resumption and the starting of projects. There is national

concern about the burgeoning costs of health care delivery. The national resources must be used to greatest effect, guided by cost/benefit and by alternative considerations. Some definitive methods are so very expensive in direct costs and in personnel costs that it is not practical to require their use in every qualified clinical laboratory. On the other hand, harvesting the benefits of such methods (e.g., isotope-dilution mass spectrometry) as definitive methods, is possible, and can be carried out for all by a designated member of the NRS. Also, the validation of materials and methods might be accomplished for all by one or more designated members of the NRS, as selected by the coordinator for each specific task. There must be quality assurance activities, a measurement assurance program on a national scale in order to achieve meaningful results in clinical laboratories under normal operating conditions. There must be mechanisms to assure long-term integrity of the measurement system - interlaboratory trials, proficiency tests. Reference materials and methods must be available for use. There must be a repository of information and materials as a part of the NRS.

Repository of Information and Materials

There must be assurance of archival permanence and be accessibility to professionals and other users of the scientific base for measurements in clinical laboratories. One way to accomplish this is to establish a central repository of reference information and materials, under the direction of the coordinator.

Do we need a central place to establish performance criteria, to certify or approve materials and methodologies, to evaluate procedures among candidate methods, to identify poor materials and procedures, those that are no longer useful or which have been supplanted by better materials or methods? This is an option; is it worth the cost? Or, can some tasks of the NRS be carried out most efficiently and effectively at a delegated place or places and others at a central laboratory?

Mandated Governmental Responsibilities

There are mandated governmental responsibilities which

must remain in place but which can be coordinated with the NRS.

THE MATERIALS COMPONENT OF THE NATIONAL REFERENCE SYSTEM (NRS)

Standard Materials

Standard materials are divided into two categories: (a) known purity standards, materials with independently verified purity and composition for which specific properties have been well characterized with respect to accuracy, and (b) arbitrary standards with assigned quantities of specified analyte substance. For arbitrary standards, the methods of preparation or assignment of numerical values are defined arbitrarily and are carefully described. Criteria for acceptance of arbitrary standards must be fully described.

Nomenclature

Proposed nomenclature for standard materials includes too many terms. There remains a lack of consensus on those which are needed and their definition. The model for the NRS will determine which terms are needed. Terms used in position papers and terms recommended by various national and international organizations should be discussed at the Conference and recommendations of the NRS be made (see definitions Addendum). The adjectives "primary" and "secondary" should not be applied to NRS materials.

Purposes and Uses

NRS standard materials should be for use with reference methods, for the evaluation of the accuracy of other methodologies, and for the evaluation of other standard materials. When available, known purity standard materials are preferred. If such materials are not available, arbitrary standard materials are developed and used. For each NRS standard material, a description of the specific uses for which it is judged suitable by the NRS and the details of use that constitute valid use should be provided with the material. Known improper uses or limitations should be stated as well. Shipping and storage conditions should be included in the labeling. For each methodology, all of the

standard materials needed should be identified.

Characteristics

The primary basis for setting the required characteristics for each standard material should be that the most important properties of the material are (1) the amount of analyte present over the time it is used, (2) other substances present must not degrade its use to measure or to compare accuracy, and (3) details of the manufacturing process must be available to assure replicate lots in the future. For each material, estimates of its characteristics, including confidence limits and the methods of establishing them, should be given in the labeling. To foster widespread use, an aim for development is to produce an adequate standard material at the lowest cost.

Biological Reference Materials

There is in clinical chemistry a lack of specimens simulating biological materials in known states of purity. There are national needs for the adoption of suitable uniform biological reference materials to measure performance characteristics, especially accuracy.

The utility of a biological reference material for an analyte is (1) to monitor the performance of a methodology over a long period of time, (2) to allow methodology transfer from one laboratory to another, (3) to be the major means of assessing performance in interlaboratory proficiency testing, (4) to evaluate performance of test methods, and (5) to provide a basis for calibrating test methods.

A definition of biological reference materials should be made. As a beginning, in order to distinguish biological reference materials from standard materials and from control preparations, we suggest the following. Biological reference materials should contain the analyte in question in a suitable matrix so as to simulate patient specimens. Although a biological matrix identical to the specimen matrix (e.g., human serum) is usually the easiest to obtain, other considerations such as cost, availability, or stability may make animal materials or synthetic materials more practical in given situations. In common with standard materials,

biological reference materials are used to assess accuracy. While a standard material could have a biological matrix, it is not intended to be specimen simulating. While standard materials are not supposed to contain interfering substances, typical interfering substances might be deliberately included in specimen simulating materials. What is meant by "specimen simulating" must be defined for each material as it applies to that material. It is probable that panels of biological reference materials would be needed, rather than one for each analyte, in order to represent the various important differences in patient specimens, such as differences in analyte concentration, disease state, and methodology interferences. In common with all reference materials, the stability of the material is very important and one must be able to repeatedly manufacture it in suitable quantitites. Details of preparation and of the means of determining concentration or amount of analyte present should be clearly described.

While the primary basis for setting priorities is the effect of the particular analysis on the welfare of the patient, there are specific considerations. These include cases where it is particularly difficult to obtain patient specimens because the prevalence of the disease state is very low, the danger or difficulty of collecting specimens from the patient is very high, or one or more component of the specimens is unstable. The current trend toward very restrictive requirements for patient consent is also causing difficulty in obtaining specimens of appropriate composition. Also, a large number of specimens may be required to evaluate a variety of methodologies based on differing scientific principles. Furthermore, the availability of well-characterized biological reference materials may eliminate or may reduce the need for every laboratory evaluating methodologies to analyze all specimens by a reference method as well as by the method being evaluated.

There must be criteria for deciding that a biological reference material is in fact specimen simulating for the intended uses. Choice of candidate materials must be very carefully considered. One must consider the analyte to be measured and the matrix of the specimens to be simulated; interferences; physical properties such as viscosity; and packaging, storage, and shipping conditions. In addition, it

must be stable and be available in sufficient quantities. When available as a stable starting material, a pool of human specimens to which various components may be added is most likely to simulate the specimen matrix. When pools are unavailable or unstable, then choices of animal or synthetic materials must be very carefully characterized to assure that they do in fact simulate the human specimen matrix. In any event, any biological reference material must be well characterized by a variety of analyses and tests.

The amount of material to be prepared relates to the stability of the material, the intended use and the number of users of the material and the facilities available to store, ship, and assure the continued integrity of the material. It would be desirable if very large amounts could be prepared at a reasonable cost so that the material could be used for calibration. Procedures for analyzing the reference material for composition, including analyte concentration and designated impurities and interferences and criteria for acceptance, must be described for each material. The question of renewing the material should be considered, particularly if it is desirable to exactly replicate the original biological reference material.

The labeling information provided with each of the biological reference materials should describe to the intended user how the material is intended to be used and assure him that it was properly prepared, tested, and stored, and that it does simulate the designated type of patient specimen.

THE METHODOLOGICAL COMPONENT OF A NATIONAL REFERENCE SYSTEM (NRS)

Definitive Methods

What is a Definitive Method?

As a basis for discussion, one might consider the definition given by IFCC for a definitive method, "A method which after exhaustive investigation is found to have no known source of inaccuracy or ambiguity." Note that it is assumed that reference materials are available which can serve to define unambiguously the analyte concentration. The

NBS definition adds a criterion for limiting testing, that the accuracy must be adequate for its stated end-purposes. The FDA agrees with this pragmatic approach to the definition.

Purpose and Function

The main purpose of definitive methods is to assign values which represent the best estimates of "true" values so that the accuracy of methods including reference methods can be assessed relative to a "true" value. The availability of samples with analyte values assigned by using a definitive method provides a means for establishing the magnitude of any bias associated with methods being considered as candidates for a reference method. After the bias is quantified to demonstrate that the magnitude of the bias is sufficiently small that the candidate reference may be used legitimately as a means for comparison, it is possible to assign priorities to other performance characteristics such as precision, specificity, sensitivity, and transferability.

Candidate Definitive Methods

The selection of a candidate definitive method should be based mainly upon meeting required performance characteristics, especially those of specificity, precision, and accuracy. These limits are set by an appropriate group of experts. The method must be well understood so that errors are assessable and correctable. At the same time, any candidate method should have a likely potential of being developed to a point such that the definition given above applies. Questions regarding the sophistication of essential instrumentation, the training of personnel, and the sensitivity of candidate methods should be secondary to the importance of accuracy and precision characteristics. Thus, large specimen volumes and the inclusion of purification steps would lead to more time-consuming procedures. There are, of course, limits. It would seem inappropriate to develop a definitive method with prohibitively large specimen volume requirements or with instrument or personnel requirements so stringent that transferability of the method is entirely precluded. Although the capability of performing the definitive method need not be available in more than one laboratory at the time of development, the reasonable

possibility of this capability in more than one laboratory is certainly desirable. The performance characteristics of candidate methods should be such that they could be reasonably developed to the point where the method would provide results having error components of sufficiently small magnitude that they provide clinically meaningful measurements. Thus, a candidate method should not be excluded from further consideration solely because there exist other methods with less precision error or because other methods are more sensitive. However, the definitive method should not be so imprecise as to provide a value with a confidence interval so large as to be clinically unacceptable and it should not be so insensitive as to preclude measurements in the low ranges encountered clinically.

Validation of Definitive Methods

The performance of a definitive method should be validated in a manner similar to that desired for a reference method. This would include investigations over clinically encountered analyte concentrations of samples representing pools of various specimen types. Ideally, a definitive method would be matrix-independent, but this characteristic must be demonstrated. Such investigations appear to be the only way to test for specimen-specific interferences. The observation of the effect of the presence of drugs or other materials with chemical structures or chemical properties likely to affect the method should be part of the validation of the performance of the definitive method. While not essential, as we have indicated above, confirmation of the reported performance characteristics by an additional laboratory is very desirable. Prior to extensive testing, there should be a statistical experimental plan. This would include (1) a description of the statistical model, (2) the experimental design (how data is to be collected), and (3) a description of how the statistical analysis is to be performed.

Definitive Methods and Reference Methods

It is apparent from the discussion above that some guidelines are needed for procedures to be followed in determining the performance characteristics of definitive

methods, as well as reference methods. Appropriate statistical protocols should minimize any concerns based upon the fact that for definitive methods only one laboratory may be examining the performance properties.

Certain assumptions have been made about reference methods in this discussion. For example, it is assumed that a candidate method will not be designated a reference method until such time as discrepancies with the candiate method and definitive method are considered to be insignificant relative to the requirements set by a group of experts. Thus, by comparing performance of a reference method with that of the definitive method, one has an estimate of the quality of the reference method result. Accuracy, precision and specificity can be measured and all other methods can then be assessed relative to a reference method, the performance of which has been characterized by comparison with a definitive method. Advisory committees to the FDA have recommended this approach.

Proposed Definitions

In the process of developing a performance standard for devices used for the measurement of glucose in serum or plasma, the FDA has followed procedures similar to those being discussed in this position paper. Specifically, the performance characteristics of a reference method have been determined by comparing results obtained with a definitive method on the same samples. Specifications for the desired performance characteristics for both methods were developed by an advisory committee composed of physicians, clinical chemists, an industry representative, and a consumer representative. During the development and revision of the standard, it became evident that there was a need to define some terms to assure that the intent of the document was clear from a scientific as well as a legalistic standpoint. IFCC recommendations served as the basis. The following useful definitions are proposed to the National Reference System (NRS) for consideration. Accuracy is a performance characteristic, measured as bias relative to a reference. Control material is a material simulating the kinds of specimens encountered in an analysis which is used to detect changes in the performance of an analytical system. It is used in statistical quality control procedures. It is not

used for calibration.

Reference Methods

The purpose of reference methods is to provide an agreed upon basis for comparing the results obtained by other analytical methods. We propose that for the National Reference System (NRS) a method be considered a reference method only when its performance is established by a definitive method and when it also meets the requirements set forth in the next paragraph. Until there are both definitive and reference methods for a given analyte, the basis for comparison shall be a provisional reference method. A provisional reference method can function as an agreed upon basis for comparison of other methods in the interim until it or another candidate can be designated as a reference method. All three classes of reference methods, provisional, reference, and definitive, are arbitrarily selected and agreed upon by NRS. If it is readily implemented, a definitive method may be used as a reference method since in this case a separate reference method would be superfluous.

In addition to having a performance that has been established by a definitive method, an NRS reference method should have the following characteristics. For a particular analyte, over the clinical range of analyte concentration, performance limits would be set as agreed upon. In practice, there will be various tradeoffs in arriving at the relative, overall merits of the candidate reference methods since their relative ranks may differ for various performance characteristics.

(1) Known and predictable bias. The bias, i.e., the difference between the limiting mean value obtained by the reference method and the limiting mean obtained by the definitive method, must be known.

(2) High precision is measured by the extent and mode of variation of successive observations about the average value. Precision must be good relative to the most precise other method to be compared.

(3) Well-characterized specificity. Ideally the specificity of a reference method should approach

that of a definitive method.

(4) Transferability. A reference method must have transferability. Transferability is achieved by the delineation of all significant and critical factors, the optimization of conditions and the designation of tolerances, and the provision of specifications and tolerances for methodology elements, including instrumentation. When possible, the method should be "forgiving." That is, the performance should remain satisfactory over reasonable ranges of values of experimental conditions. Transferability must be validated by interlaboratory testing.

(5) Reasonable implementability. There must be reasonable time constraints; capability to analyze adequate numbers of samples without sacrifice of performance characteristics; tasks assembled into interruptable sections, the largest of which should take no longer than one working day. There should be, when feasible, a minimization of input from highly trained and experienced professionals.

(6) Designated computation method. There must be designated appropriate units for expression results, technique for mathematical computation with examples, and rules for outliers.

(7) Small sample size capability is desirable, though of secondary importance.

(8) NRS reference materials should be used.

(9) There should be identical treatment of specimens and standards.

The validation of a reference method is based, first, upon establishing its bias, precision and specificity by comparison of results with those obtained with a definitive method. There should be a rationale for observed bias. The effects of interfering substances and conditions need to be known. All of the analytical priciples, procedures, apparatus, and materials must be well characterized and

understood. There must be a description of methods to determine if the reference method is out of control, e.g., quality control techniques. All significant details of the method must be carefully described so that the method can be exactly repeated by all who use it.

The selection and adoption by NRS of nationally agreed upon reference methods should be guided by the intended uses:

(1) as the accepted means of assessing bias and precision, specificity and sensitivity of methologies and/or in vitro diagnostic devices,

(2) among laboratories, to assess transferability of selected methodologies,

(3) to provide a common base against which to express a product's or methodology's performance,

(4) to assess the accuracy of the assigned values for analytes in calibration and control materials,

(5) to establish or verify reference values or ranges.

Setting priorities is an important function of the NRS. The selection and assignment of priority to analytes made by the Advisory Panel to FDA for standards development can be considered by the NRS. This priority has been primarily on these experts' understanding of the medical need for reliable diagnostic information, on their experience that available diagnostic devices do not uniformly provide this reliability, and on the availability of appropriate candidate reference materials and methodology.

The selection of a reference method should be made assuming its implementation in a laboratory with instrumentation and personnel adequate to maintain the analytical integrity of the method. It should be selected with the understanding that the reference methodology is not intended for routine use, and the choice of method should not be compromised for convenience. However, the methodology should not represent such complexities that its use by well-equipped and capably staffed laboratories is precluded. Furthermore, the use of reference methodologies in

comparative studies with other methodologies or products which require sampling protocols of numerous specimens and multiple runs should be borne in mind when making a selection.

The organization which undertakes the development and validation of a selected candidate reference method should be able to satisfy the need for access to appropriate specimens, for access to medical information on abnormal specimens, and for appropriate instrumentation and properly trained personnel. Also required are adequate resources to undertake the comprehensive studies needed to optimize experimental parameters, to perform interference studies, and to assess the limits of performance of the method with respect to accuracy, precision, specificity, and sensitivity. There must be the capability and resources to demonstrate reference method transferability to other laboratories which are suitably equipped and staffed. This requires coordinating the dissemination of the reference method, providing materials, specimens and controls as necessary, evaluating participating laboratories' performance, assessing the transferability of the method, and establishing criteria for successful transfer including any quality control procedures to insure integrity of the reference method.

The promulgation and maintenance of an NRS reference method should provide for periodic monitoring of the performance of participants (on a voluntary basis) by supplying appropriate specimens and by assessing results to determine the continued integrity of the reference method, to insure maintenance of adequate levels of performance by participating laboratories, and to provide a means for new participants to institute the reference methodology or previous participants to re-establish the reference methodology capability.

Provisional Reference Principles

In view of the fact that reference materials and methods are not universally available (but in some cases are under development to a very limited extent), provisional reference materials and methods would fulfill a practical need for the interim until better materials and methods are developed, which are considered to be of reference grade. In order to

effectively evaluate the performance of in vitro diagnostic devices, particularly accuracy, a sound basis of reference must be available. Ideally, a fully validated reference method with associated reference materials would fulfill such a need. In the absence of fully validated reference materials and methods covering the scope of products used in the clinical chemistry laboratory, provisional reference materials and methods would be a vital step in the right direction.

The FDA supports the development of provisional reference materials and methods because such an effort would result in the later development of a sound technical basis to be used for the evaluation of marketed in vitro diagnostic devices. Additionally, the availability of provisional reference materials and methods would accelerate the development of validated or non-provisional reference materials and methods as a basis for judging accuracy and overall performance of in vitro diagnostic devices. Such evaluations may be in connection with determining compliance with a performance standard or to determine the validity of a manufacturer's performance claims on the device's labeling.

The development of provisional reference materials and methods would require the use of an approach generally agreed upon by the clinical laboratory scientific community. Such an approach would have to focus on whether consensus is necessary, if so, how it would best be obtained. Additionally, the term "consensus" is in need of definition. It is the position of the FDA that acceptability of provisional reference materials and methods should include in its process, a supporting rationale, fully documented. The documentation would describe the materials and method considered and the reasons for a particular material or method being selected, as well as the reasons for the rejection of others.

The provisional reference materials and methods should serve (1) as the agreed-upon basis for comparing performance characteristics in the interim until there is a reference material or method, and (2) as a candidate reference material or method.

A required characteristic of a provisional reference

method is high reproducibility. There should be a lack of unexplained discrepancies with other arbitrary reference methods. In the absence of a definitive method, a provisional reference method should have as many as possible of the other characteristics of a reference method.

ADDENDUM: DEFINITIONS

As a response to the letter dated June 29, 1977 from David D. Bayse, Ph.D., to the Conference Participants, this addendum regarding definitions has been prepared for our Position Paper.

We are opposed to the adoption by this Conference of the glossary in the AACC Position Paper as the definitions to be used in position papers and other Conference communications. We are aware that this AACC glossary is a worthwhile step toward improved definitions. We find that some of the definitions require modifications and so we do not recommend the adoption of this nomenclature in its entirety.

Although a set of definitions for the NRS would collectively constitute a description of the NRS, we believe that a more consistent, complete, and rational set of definitions would result from starting with the to-be-agreed-upon model or framework for the NRS. Whatever definitions are needed should then be added. The idea of working independently with the definitions which are elements of the NRS and then putting them together later as a model is not as effective. Moreover, the definitions are also essential expressions of Analytical Goals and Quality Requirements. There should be a joint effort of several groups rather than an effort by any group working separately. In addition, we feel that major attention should be concentrated on designing the model of the NRS as such and that the definitions should follow and support this model. It is essential that the NRS definitions be consistent with each other and with the model.

Also, we believe that all of the definitions found in the various Position Papers should be given due consideration. At this date, we have only a portion of the Position Papers at hand. We think that there is a need to devote some time at the Conference to a discussion of the

model of the NRS and that the model needs to be agreed upon before definitions are discussed. We also request that consideration be given to definitions proposed in the FDA Position Paper and in all other Position Papers as well.

Specific Comments (Glossary Definitions)

Accuracy. The proposed definition is inadequate because "...the best estimate of a quantity..." can be interpreted to have widely different meanings. Also, the FDA position is that accuracy allows for various qualities of an estimate of accuracy, in that bias may be estimated by whichever class of reference is stated to be used; provisional reference, reference, or definitive. See the FDA definitions of "true" value, bias, accuracy and medical accuracy.

Aliquot. Must be changed to "fraction" rather than "portion". An aliquot is an exact divisor or factor of a quantity.

Consensus. See FDA definitions of "consensus" and of "majority opinion."

Control material, specimen, solution. Insufficient detail. See FDA definition of control material.

Error. See FDA definitions of "true" value, accuracy, bias, and medical accuracy. The limiting mean values should be used, not the arithmetic means.

Imprecision. No new information is achieved by adding "im" to "precision" or "in" to "accuracy".

Interference. A substance or a condition causing interference is not necessarily a compound. A more general term must be used rather than "compound" e.g., "substance."

Material, reference. See NBS definition.

Method, definitive. See NBS and FDA definitions.

Method, reference. See NBS and FDA definitions.

Method, special reference. FDA has adopted CDC/NRS terminology. See FDA definition of provisional method.

Run. We note that a definition is necessarily protocol dependent. We propose that the results are always calculated from the same set of calibration values.

Sensitivity. Has another meaning, in addition to detection limit, namely, discrimination or resolution, e.g., for qualitative interpretation purposes. Does the NRS wish to include both meanings?

Systematic error. See FDA definition of bias.

INDUSTRY

POSITION PAPER

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Preface

In this position paper, the representatives of industry have approached the overall topic in a manner that may differ markedly from the other participants. It should be understood that the industry may not perceive the development of reference methods and materials in the same ranking of priority as the other groups which also tend to have a more closely defined interest in the subject. For example, the industry is, at various times, both a consumer of reference methods developed by others as well as a transmitter of reference materials through commercial manufacture and distribution. Perhaps more importantly, the industry is the essential source of the routine technology and materials that are the foundation of laboratory performance from the smallest to most sophisticated facilities. Also, the industry takes pride in the fact that research and development into commercially feasible products has enabled the laboratory community to avail itself of increasingly better and more useful diagnostic capability. These characteristics of the industry have influenced the contents of this position paper. Accordingly, the position paper demonstrates a conservative approach to a national understanding objective. Industry has attempted to emphasize suggested principles of understanding rather than specifics of organization, methods and materials. Lastly, the industry will measure the success of the national understanding conference in terms of constructive decisions or agreements that could enhance the reliability of results in day-to-day laboratory performance rather than advancement in theory that may lack practical translation or application.

The industrial sector must also stress that it does not possess the capacity to represent and speak for the multitude of manufacturing firms that could eventually participate in a national reference system. Although obvious, it must be reiterated that the contributors to this document, as well as those companies that have reviewed it, are frequent business competitors of each other. Thus, it would be unrealistic and most likely impossible for an industry position paper to be all-inclusive or reflect unanimity on attitudes expressed or on the details of specific approaches to reference method or material development. Similarly, the participation of trade associations in this document takes the form of coordination

and dissemination as opposed to any form of official or binding endorsement. In order to be most constructive, therefore, the position paper represents a cross-section of industrial views that are offered for consideration and discussion at the National Understanding Conference. Acordingly, the free enterprise nature of the industry, including the clear right of individual company interpretation and expression, must be recognized during the review of this paper and during the Conference deliberations.

INTRODUCTION

In the interest of improving health care, a common reference system of relevant clinical chemistry measurements should be instituted to serve the diversified needs of the health care community. The elements of the health care community are recognized as being (1) the clinical community (i.e., physician, laboratorian and patient); (2) the agencies of government; (3) academia and (4) the industry which manufactures measurement systems and supplies services.

This system must provide for the development and acceptance of reference materials and methods and ensure their applicability to laboratory practice. It should provide, in public forum, a process to include identification, priority designation, development, review, confirmation/validation, transferability and endorsement of reference methods and materials. The issue is whether developments in the clinical laboratory field will continue to evolve on a random basis or whether priorities and programs will be given direction and purpose based on national health care requirements. We believe that standards can be developed on a voluntary basis with the elements of the health care community participating in an open consensus environment. Further, we are confident that these standards are attainable by employing a stepwise progression of agreed upon objectives or "building blocks". These building blocks would relate to those items that are more or less immediately attainable; those that can be attained in the near term; and those which require more careful study for the longer term. As an example of what can be achieved immediately, even before the Conference is adjourned, is the description of the mechanism for adopting and endorsing reference methods and

materials. An example of a near-term objective would be the procedure for establishing medical goals and priorities as has already been initiated at the Aspen Conference in 1976. A longer term objective would be the definition and implementation of an umbrella organization to supervise and administer the initiatives, efforts, changes and expansions required to eventually institutionalize the national understanding goal.

The motivation for beginning the formation of a National Reference System (NRS) stems from the recognition that the future of clinical chemistry is clearly tied to the development of improved laboratory services. It should be recognized that the quality of these services to date has steadily improved through the efforts of many organizations and individuals with generally compatible objectives. Thus, it is appropriate to provide the interested parties with a broader array of tools and a more cohesive network to move from unilateral to multilateral undertakings.

The NRS should be designed to draw on the strengths of each contributing element based on its competence and natural incentives. However, at the outset, the industry is concerned that the purpose and role of the NRS must be thoroughly explored and defined so that no one organization or sector of interest would inappropriately influence the direction of the effort. For example, the output of the system cannot be preferentially used to satisfy product regulatory interests that could produce stricter regulation and manufacturing demands without regard for medical relevance. If so, the clinical diagnostics industry would then experience, to no one's benefit, escalating research and development costs resulting in a significantly decreased rate of introduction of new _in vitro_ diagnostic products. Of course, the industry recognizes that certain arbitrary decisions (i.e., those lacking a scientific base) may be made and can be justified based on expediency as long as they relate to objective and relevant clinical goals. What must be vigorously avoided is any irresolute or parochial interest that resists compromise within the workings of the system.

It should be understood and appreciated that an ill-conceived or misdirected standards effort would, despite the best of intentions, reduce innovation. This is of

particular concern to the field of clinical chemistry which is undergoing rapid advances in technology and methods. Therefore, the most important objective of reference materials and methods should be the quality of the answer--not the manipulation involved in obtaining the result. Also, any proposed reference system should include a simple built-in mechanism for continuing evaluation of methods and then supplementing or replacing them as better ones become available.

When developing a reference system, the ultimate use of the test and its clinical significance must be considered when performance limits are set. Moreover, the requirements of reference methods should be clearly distinguished from the requirements applicable to routine laboratory methods.

Taking into account the fundamental issues discussed above, it appears that the interests of the full health care community could be accommodated by an organization that has both resources and objectivity and can grow on a timely basis to meet the needs we have outlined. A clear example of such an organization is the National Committee for Clinical Laboratory Standards (NCCLS).

ANALYTICAL GOALS AND QUALITY REQUIREMENTS

The industry holds the view that the health care community should not become preoccupied with analytical goals at this initial stage of development of the NRS. While general conclusions on analytical goals should be framed it would be ill-advised to concentrate definitive effort in this area until more fundamental aspects of the NRS, such as agreement on methods and materials qualifications, are in place.

In the past, great effort has been expended to make laboratory data more accurate and precise. The evolution in this area has been successful based on the natural incentives of participating organizations and individuals and it is the opinion of industry that it will continue to be successful without the involvement of a new organization or the imposition of a master plan unless fundamental and feasible accords can be reached and supported by all interested parties.

The proposed NRS is in fact the multidisciplinary recognition of the desire to formalize a set of relationships that have evolved over the years between practitioners of laboratory medicine, agencies of government and industry. Conceivably, there should be no change in either form or content from what has gone on in the past. Perhaps the most salutory effect in bringing all the parties together is continuing dialogue and the opportunity for focused and directed energy to achieve agreed upon goals.

At the center of the question of accuracy and precision goals for the development of materials and methods is perhaps an even more fundamental issue. How is this already accurate and precise data being utilized by the consumer-physician? It does no good to produce more accurate and precise data if presently available information is not being used to its full potential. One suggestion is that the scope of the NRS might include a discussion of methods of information transfer. The time has come to stress the interpretation of the information as much as the quality of the data.

The discussions at the Aspen Conference of 1976 were valid in focusing attention on the variables which affect the quality requirements of testing procedures. However, not all the variables were identified. The entire discussion related to univariate data, whereas, in real life most testing is multivariate. It seems appropriate that the NRS should extend the work of the Aspen Conference to a truly comprehensive scheme for setting goals.

As pointed out by the participants at the Aspen Conference, there is need for a detailed model to describe the simultaneous effects of bias (inaccuracy) and imprecision on sensitivity, specificity and apparent individual variation. Such goals should be stringent enough to assure diagnostic confidence, but not arbitrarily small for no practical reason. Therefore, a potential goal of the NRS would be to develop a scheme for setting goals based on knowledge of the variables involved and the nature of the clinical use of the laboratory data and its information content. Clearly, this goal requires extensive collaboration among numerous interested parties and should, therefore, be assigned as one of the desirable but longer range objectives of the NRS.

Additionally, it is important to recognize as pointed out in the forthcoming Aspen Conference Proceedings, that "no single criterion has been able to answer satisfactorily the increasing demand by consumers, regulatory agencies and instrument manufacturers to document standards of quality performance".

It must be reemphasized that the only basis for setting goals for the development of methods and materials must be defined in terms of patient care. Only after the medical goal has been clearly stated will it be possible for each element of the clinical community to respond on a rational cost effective basis. Thus, industry representatives believe that the criteria for goal setting are stated in a key observation of the first of the Aspen Conference Recommendations.

Aspen Conference Recommendation #1
(Excerpt)

"Analytical goals can only be defined in terms of the needs for patient care. Any other basis is irrelevant."

The priorities of any organization or authority for the development of reference materials and methods must be measured in these terms. As noted earlier, this recommendation involves an extensive commitment of time and collective organizational resources. Accordingly, the industry sector advocates pursuit of the analytical goal as a long term goal to take a lesser priority to the establishment of the basic framework of the NRS. An effective mechanism for achievement of more immediate goals at the Conference should be our first priority.

ORGANIZATIONAL COMPONENT OF THE NATIONAL REFERENCE SYSTEM

The health care community is recognized as being a multi-disciplined combination of constituent professions, organizations, and industrial corporations with diversified interests. These elements can broadly be described as: -- medical service community; -- educational and research community, termed generically as academia; -- governmental sector; and -- industry, or the manufacturers who serve the needs of the first three elements.

Each element is characterized by its particular discipline and self interests. All, however, have a common goal of providing better health care. Because of the diversified interests, each element uses different criteria to judge success or failure. All agree, however, that they must use a common system of clinical chemistry measurements and it is clear that the charter of this Conference is to define the development process and explore the possible contribution that each element can make to the process. Acknowledgement and commitment to this process will be known as the National Understanding for the Development of Reference Materials and Methods for Clinical Chemistry.

A proposed pathway for processing candidate materials and methods for use in the NRS is illustrated in Figure 1.

The steps in Figure 1 represent three phases of the national understanding process. The first two steps relate to setting medical goals and priorities. Even though these steps appear at the beginning of the process, it must be recognized that the chart depicts the process in full operation--a development that the industry does not believe to be immediately realizable. Assigning the medical goals as a near term objective, the industry proposes to concentrate on the more immediate achievements which are reflected in the remainder of Figure 1. Set forth in the chart is a mechanism to adopt and endorse reference materials and methods using the presently available inputs for the system. The NCCLS could administer the process and it is suggested that the conference participants debate and test the proposed procedure to determine whether NCCLS offers the best organizational resources for the task. In all likelihood, the procedures presently in use by NCCLS would require some modification and expansion to successfully accommodate the proposal especially since NCCLS is not currently involved in validation or transferability functions.

The major elements in this proposed scheme are:

1. Establish medical goals and priorities

2. Develop reference methods and materials according to priority

SUGGESTED PROCEDURE -
UNDERSTANDING FOR THE DEVELOPMENT OF REFERENCE MATERIALS AND METHODS

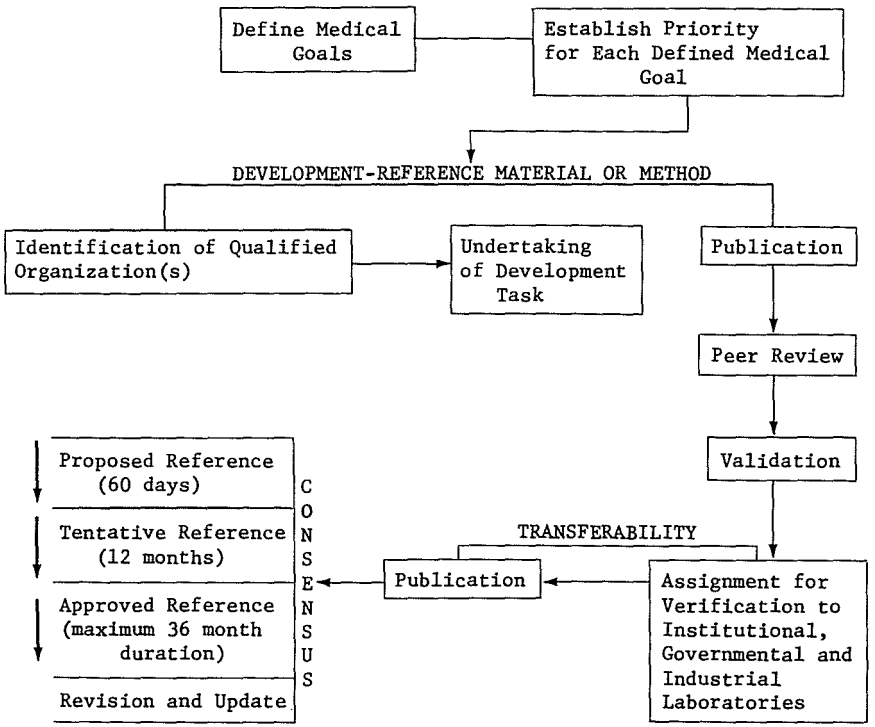

Figure 1

(The scientific literature will provide steps 1 and 2 with open discussion and peer review, which are essential to their successful development.)

3. Validate the reference materials and/or methods

4. Verify the transferability of the reference materials and/or methods

5. Invoke the consensus process

Shown in Figure 2 is an example of logical contributors to each step in the process, based on discipline, resource and self interest. It must be understood that this designation of elements in the health care community is loosely defined and that component organizations may contribute through more than one element. A professional organization such as CAP or AACC may in one case be regarded as academia and in another case as the medical community.

The medical community has earlier described[1] goals and priorities in the laboratory. Academia as well, has recognized[2] the deficiencies in the laboratory practice and made recommendations to improve the practice. The governmental regulatory community could join these two and arrive at an updated priority list to suit its own needs for regulatory effectiveness.

The Conference should generate an understanding by the health care groups that each element of the complex is prepared to participate to achieve a common goal. The National Reference System thus is an open process with opportunities for peer review, publication in the medical-clinical literature, consensus approval and periodic review of accomplishments.

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[1]Barnett, Roy No., Am. J. Clin. Path. 50, 671 (1968)

[2]Tonks, David B., Clin. Chem. 9, 217 (1963)

FIGURE 2

Illustration of Responsibility and Tasks of
the Involved Elements in a National Reference System

	Medical	Academia	Government	Industry
Define Medical Goals	●	●		
Establish Goal Priority	●	●	●	
Develop Reference				
-identification of organization	●	●	●	●
-undertake task	●	●	●	0
-publish results	0	●	0	0
Peer Review	●	●	●	●
Validate Reference	0	●		
Demonstrate Transferability				
-assignment for verification	●	●	●	●
-publish verification	0	●	0	0
Consensus Process				
-proposed reference	0	0	0	0
-tentative reference	0	0	0	0
-approved reference	0	0	0	0
-mandatory revision process	0	0	0	0

● Primary Responsibility

0 Secondary Responsibility

THE MATERIALS COMPONENT OF THE NRS

Standard Materials and Biological Reference Materials

There are two basic needs for reference materials in the National Reference System. In one case, reference materials may be necessary to calibrate the reference method. In general, the calibration reference materials will be equivalent to SRM's. In addition, for effective transferability testing, control reference materials (CRM) may be needed. The control reference materials will, in general, be sample simulating materials assayed by the reference method in a control laboratory. These CRM's will have limits of uncertainty based on actual assay.

There are several types of reference materials which are needed. These include materials which can be defined by elemental composition, (e.g., NaCl); materials which can be defined by chemical composition and physical properties to specify purity levels, (e.g., bovine serum albumin); and materials which can only be defined in terms of their biological activity, (e.g. enzymes). Each of these can exist in pure form or in complex matrices.

The following goals and guidelines should be addressed and met at the forthcoming conference with regard to the development of reference materials:

1. To continue and accelerate the development of standard reference materials, which, because of the similarity to existing SRM's, will be called SRM's in the balance of this discussion. These SRM's should include pure (both organic and inorganic) and biological materials represented by proteins, other polypeptides, nucleic acids, etc. as well as analytes in a real or simulated biological matrix.

2. To maintain and assure the constant availability of SRM's. Even short term unavailability of reference materials would place the usefulness of the entire National Reference System in a difficult position.

3. Each individual SRM must be characterized with regard to the type of testing required to qualify the material

as an SRM.

4. The limit of uncertainty of an SRM/CRM must consider:

   a. the stability of the analyte

   b. inter-vial precision

   c. homogeneity

   d. known impurities (pure SRM's)

   e. the structural complexity of the analyte molecule

   f. the matrix effect of any biological SRM/CRM as it would relate to the equivalent testing of fresh human serum.

5. SRM's/CRM's must be in a form that is suitable for the intended use. Minimal manipulation of the SRM to make it ready for use is a mandatory requirement to prevent adulteration.

6. Any group or organization should be allowed to develop candidate SRM's/CRM's for appropriate certification.

7. The consensus concept of establishing a quantitative value for a SRM/CRM should not be used.

8. That only materials that have been certified as SRM's may be used to standardize reference methods.

The use of pure biological materials offers the advantages of: 1) the opportunity to characterize and define a material; 2) freedom from interfering substances which complicate the analysis; and 3) the ability, in most instances, to be prepared in quantities sufficient for use over an extended period of time. Disadvantages of the use of isolated and purified biological materials are: 1) isolation may change some important characteristics of the molecule; 2) material may no longer be similar to that found in samples which are to be used with a candidate reference method; 3) instability; where some pure materials are often far less stable than native biologicals; 4) matrix effects; where

purified materials matrix effects must be delineated and then performance related to the identical component found in a body fluid. This means, in a practical sense, that each biological SRM is the subject of an expensive project prior to its qualification.

For those materials of biological origin where it is impractical to isolate the analyte for preparation of an SRM, assay of material in a serum pool may be the only means of accomplishing the goal. Some disadvantages of using serum pools as a source of analyte for use with reference methods are: 1) the possibility of interactive association may preclude precise standardization of a reference method, and 2) the dependence on method; the analysis of or the performance characteristics of the material in a biological matrix may be defined by the method used thus complicating the comparision between methodologies.

THE METHODS COMPONENT OF THE NRS

Definitive Methods

Considerable confusion will exist in discussing a National Reference System until terms are defined and procedures are outlined. Potential resistance derives from fear of the unknown and uncertainty as to just what certain terms and procedures will mean. The revised copy of the IFCC quality control nomenclature recommendation does not provide an acceptable working definition for the purposes of this conference. Nomenclature must include the following concepts:*

A "definitive method" is an analytical method that is capable of providing highest accuracy among all methods for determining that analyte, and its accuracy must be adequate for its stated end-purposes.

A "reference method" is an analytical method whose inaccuracy and imprecision are small enough, as demonstrated

*(The definitions given are as presented in the NBS Position Paper, except for the addition of a bracketed phrase in "reference method.")

by direct comparison with a definitive method [or by other means], and whose low incidence of susceptibility to known interferences is thoroughly documented so that the stated end-purposes of the reference method may be achieved.

A "provisional reference" is a well characterized analytical approach known to achieve reliable results which will provide guidance in terms of accuracy and precision goals.

With these assumptions or definitions, the principle thrust of the National Reference System should be the development of reference methods that can be used with confidence by clinical laboratorians using the commercially available equipment. Definitive methods deserve second priority. Generally they can be best justified when the need arises to prove a candidate reference method.

Reference Methods

The principal thrust of the National Reference System should be the development of Reference Methods which have the accuracy and precision required for improved health care. These reference methods should be arrived at by using a consensus process.

Reference methods are used:

a. To assess the performance of an analytical method or product.

b. To test samples and materials that will be used for laboratory evaluation.

c. To evaluate materials used for the routine quality control of the laboratory.

d. To assign values for calibrators.

Reference methods must be applicable for use with routine clinical specimens in that a significant number of patient sera must be analyzed in parallel by both the method under evaluation and the reference method in order to expose potential interferences not previously suspected.

Generally, a reference method would not be used for routine clinical work. A reference method must be understood to be complete in detail. It is also obvious that it would be inappropriate to claim for commercial purposes that use of the methodological principle of a reference method implies that the reference method itself is being used.

It is probably premature to address specifically the following topics during the NRS conference in Atlanta, although they must be addressed at a later time:

(a) Quantitative limits on precision and accuracy for specific clinical tests.

(b) Priorities for the development of reference methods or materials.

(c) Proficiency testing or the clinical basis of proficiency data.

(d) The preparation of calibrators.

Of critical importance to the concept of a meaningful and responsive NRS is the mechanism for change. The performance characteristics of reference methodology will be surpassed in many instances by newer technology. Procedures must be available that permit the NRS to change or replace a reference method.

Provisional Reference Methods

Provisional reference methods are precursors to reference methods. They are, in general, not supported by sufficient information on accuracy, precision, interferences, transferability, etc., to qualify for use as reference methods.

NATIONAL BUREAU OF STANDARDS

POSITION PAPER

by

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INTRODUCTION

In this paper the components of a national clinical measurement system and the relationship of reference materials and methods to that system are discussed. It is the belief of the authors that the availability of reference methods and reference materials which have a known, and tightly controlled, accuracy base are essential to provide compatibility in such a measurement system. Accuracy is particularly important because, due to the high mobility of the population and the fact that more and more physicians are obtaining laboratory results from more than one laboratory, comparison of laboratory results can be both complex and confusing.

A national clinical measurement system must be based on a series of technical components that exist at various levels of sophistication but are tied together. These will be discussed in the sense of a general classification of methods and materials. In both of these general categories, a consistent system of units, preferably those of the System Internationale, should be used in defining concentrations and other appropriate parameters.

In the clinical chemistry community it is now generally accepted that there are three general "levels" of methods available. These are often referred to as definitive methods, reference methods, and laboratory methods and are in decreasing order of sophistication, accuracy obtained, and, generally, expense and difficulty in performance. A definitive method or a reference method and a method used commonly in a laboratory could conceivably be the same, although this would be the exception rather than the rule. The definitions and practical considerations involved in the development and use of these methods are discussed below.

Laboratory methods, or field methods as they are sometimes referred to, usually have somewhat broader range of results and are used for routine application in clinical laboratories. In a hierarchical system of measurement methods, the accuracy of laboratory methods should be, as much as possible, related directly to a definitive method, usually through a reference method.

In the area of reference materials, there are again subcategories (which are discussed in detail below). These subcategories are based on the accuracy base and/or form of the analyte for which analytical values are given. If the values are specified and certified to be accurate within a certain range by some recognized body, they are referred to as certified reference materials. Such materials may be certified by national standardization organizations, or by others. The certified reference materials prepared by National Bureau of Standards are referred to as Standard Reference Materials.

ANALYTICAL GOALS AND QUALITY REQUIREMENTS

The primary purpose of a national clinical measurement system must be improved patient care. Clinical measurements must be sufficiently accurate to satisfy these patient care needs. Over the past 10-15 years there has been an "explosion" in the number of requests for clinical laboratory tests in the United States. This has resulted in the development of automated analysis equipment for those tests requested most often. Defining single goals to be achieved in a clinical analysis system, whether by manual or automated analysis, presents many problems because the accuracy requirements for each analyte will be different. These differences are based on both biological variability and the quality of data needed for diagnostic interpretations. The problem is compounded by pragmatic considerations such as availability of methods and economic factors. A general rule of thumb could be that the laboratory method, when used with an acceptable reference material, should give results that are "as good as needed". One must be cautious in applying this rule. In spite of fairly large biological variability, significant utility is often discovered from more accurate results which was not obvious from previously obtained, relatively inaccurate data.

It should also be clearly understood that the clinical measurement system must be under constant evolution. The recognition and endorsement of standard methods by appropriate standardization bodies must not be allowed to retard the development and implementation of more accurate methodology. Provisions must be built into such a national clinical measurement system to provide for steady growth and

improvement.

To express adequately the components of a measurement system, a statistical evaluation of the accuracy desired (or obtained) must always be given. The analytical goal should take into account both analytical uncertainty and biological variability. The uncertainty of the component system should always be equal to or less than the maximum acceptable error. For example, if the system must be accurate to at least within $\pm$ 20%, $B_L \pm 2\sigma_L \leq 20\%$, where B is the bias, σ is the standard deviation, and L is a laboratory method. In this equation it would be acceptable, for example, for B_L to be $\leq 10\%$, and $\sigma_L \leq 5\%$. In general, the error acceptable for a laboratory method will be greater than that for the definitive method. The performance requirements when developing a new method, must be based on providing an acceptable error no greater than that required in the laboratory.

In most cases the uncertainty of the value of the analyte in a primary standard material should be approximately equal to that available when using a definitive method, and should always be less than the end use for which the method is to be used.

A national clinical measurement system should provide the most accurate results possible under the constraints of availability and economics. It should be developed and implemented in an atmosphere of free cooperation and communication, and to be, above all, of the greatest practical utility to the physician. This will result in a greater understanding of biological function and improved patient care.

ORGANIZATIONAL COMPONENT

The clinical chemistry measurement system is large and technically complex. To be effective, the system must be highly interactive. Both technical and economic considerations must be taken into account in the design and implementation of a compatible measurement system.

With regard to materials and methods, a hierarchical approach seems to be essential. Primary and secondary

reference materials, calibration and control materials, as well as several grades of reagents already exist. Three tiers of methods also seem to be required: definitive, reference, and practical laboratory. No single organization has either the resources or technical skills to develop and implement all of the different technical components of the clinical measurement system. For the many thousands of clinical chemistry laboratories performing upwards of several billions (10^9) tests yearly, obviously require high volume industrial capacity. On the other hand, the measurement and certification of primary reference materials is technically difficult and generally economically unattractive. The development of certified primary reference materials would seem to call for the services of government supported laboratories. Analogous arguments apply to the various classes of methods, especially with regard to rapidly evolving new measurement techniques that require the attention of professionals whose activities are often focused in their societies. Given the complexities just outlined, it may be most effective to coordinate these activities through one central organization.

Economically, it is desirable that the organization designed to coordinate these activities be self-supporting insofar as practical. Both private and public organizations involved in the clinical chemical field, should support the direct organizational costs and also be willing to supply appropriate services gratis to the organization. Ultimate cost reduction should be an important consideration consistent with the stated primary technical goals.

The U.S. voluntary consensus standards system is almost unique in the world and has produced more useful and internationally accepted standards than that of any other nation. NBS is dedicated to the support and strengthening of the voluntary consensus process. This process has worked well in other technically complex fields (nuclear, e.g.) to provide fair, equitable, and technically sound standards and has also served as a basis for regulation in areas where regulation is considered necessary. NBS feels a national, voluntary consensus standards organization would best serve the interests of the U.S. clinical chemistry measurement community.

Organizational Structure

A national voluntary consensus standards organization for clinical chemistry already exists in the U.S. It is the National Committee for Clinical Laboratory Standards (NCCLS). The NCCLS Administrative and Procedural Manual and its By-Laws indicate that essentially all the necessary conditions for a truly representative consensus standards body have been carefully considered and addressed. Whether all conditions have been faithfully met in practice is not under consideration here. However, NBS will use the existing NCCLS structure as an example of how we think this or a similarly structured organization could serve to develop and implement a national reference system for clinical chemistry.

THE NATIONAL CLINICAL CHEMISTRY COUNCIL

We envision a guiding, advisory body attached to the major group responsible for clinical chemistry. (In the NCCLS, this would be the Area Committee on Clinical Chemistry.)

a. Characteristics of the Council

1. true representation of all segments of the the clinical chemistry community necessary in the development and maintenance of a national, compatible measurement system;

2. non-domination by any one segment of the community;

3. structured to allow and encourage the widest participation of knowledgeable and interested people; and,

4. true consensus in all its actions and deliberations.

b. Council Responsibilities

1. Establish, based on consensus, national priorities for work to be done at Subcommittee level.

2. Set up Subcommittees and their membership as

appropriate to establish and develop a compatible measurement system for a designated analyte.

3. Ensure through formal process the technical competence of nominees to Subcommittees.

4. Assure consensus input at all stages, through proper publicity.

5. Review work of Subcommittees and arrange for consensus review.

6. Assure wide-spread publicity for work accomplished.

7. Ensure an operational periodic review mechanism.

c. Subcommittee Responsibilities

1. Follow consensus guidelines for output of the work. This will require proper and formal documentation.

2. Adhere to the priorities set by the Council.

3. Set goals, schedules, lists of participants, etc., for all technical work to be performed.

4. Organize technical work through delegation to a specific organization, laboratory or individual. Ensure that proper documentation is developed at all stages. Such work could include: development and verification of definitive or reference methods, development of reference materials, etc.

5. Review completed work for technical validity.

6. Report plans, priorities, and work in process to the council for communication to the clinical chemistry community.

Perceived Role of NBS

NBS has certain responsibilities delineated by its congressional enabling legislation. These include the provision of primary methods and materials upon which a

national measurement system must ultimately rest. This broad legislative authority includes definitive methodology and primary reference materials. Also included is related work involving the transfer of the accuracy inherent in these primary analytical components to the user community, e.g., involvement, but not necessarily the prime role, in reference method development. Therefore, NBS would seek representation on the Council and representation on appropriate Subcommittees concerned with definitive and reference method development and primary reference materials.

MATERIALS COMPONENT

Background Information

The International Standards Organization (ISO) has tentatively defined[1] a reference material (RM) to be "a material or substance, one or more physical or chemical properties of which are sufficiently well-established to be used for the calibration of an apparatus or for the verification of a measurement method. Generally, any reasonably small part of a RM sample should exhibit the property value(s) established by the RM as a whole, within the stated uncertainty limits." A certified reference material is defined to be "a reference material accompanied by, or traceable to, a certificate stating the property value(s) concerned and issued by an organization, which is generally accepted as technically competent."

The International Federation of Clinical Chemistry (IFCC) has adopted a provisional recommendation[2] for terminology relevant to the area of reference materials. IFCC defines a primary standard material to be "a substance of known chemical composition and sufficient purity to be used in preparing a primary standard solution."

The National Committee for Clinical Laboratory Standards (NCCLS) has approved a standard for reference materials which are used for either calibration or control purposes[3]. A calibration reference material is one whose constituents are established with sufficient accuracy and precision to be useful for calibration purposes. The NCCLS standard provides that calibration reference materials have values of constituents determined by comparison to primary standards

(primary reference materials) whenever possible. NCCLS describes both calibration materials and control materials as substances used to simulate analytical samples, which are usually complex mixtures and are commonly derived, at least partially, from biological sources.

Hainline and Schaffer[4] have discussed the development and use of calibration reference materials and "controls" in clinical chemistry. They show how the former can serve as the basis for assignment of values by analysis whereas the latter serve to provide data with which the precision of analyses can be estimated.

The above definitions as well as others not referenced here (e.g., those of IUPAC) are not entirely consistent with each other. The confusion over terminology should be eliminated. In the interim, the terms shown in table 1 are those used in this paper and are recommended to the conference as a basis for discussion. The major characteristics of primary, secondary and control reference materials and their role in the clinical measurement system are also summarized in table 1. The following sections describe these materials in more detail.

Primary Reference Material

The designation "primary" should be reserved to describe reference materials which are characterized using materials processing techniques and measurement methods designed to achieve the highest possible accuracy consistent with end-use requirements. Characterization of primary reference materials should be by definitive methods (when available) and/or a number of highly reliable independent methods previously shown to be suitable for high accuracy analysis. Primary reference materials must be available for general distribution.

In the context of the above definitions, Standard Reference Materials (SRM's) are primary reference materials. The designation certified reference material should be reserved for those materials certified by a government agency, commercial organization, or professional body, generally accepted to be technically competent. The concept of certification is "legal" or "quasi-legal" in nature rather

than technical.

The term primary reference material should not be reserved to denote only a chemical substance of highest purity, but may also be used to describe a material that has been highly characterized with respect to an analyte in a matrix. In the latter case, the property value must be determined by a definitive method or a number of highly reliable independent methods. The major use of primary reference materials should be to assure measurement compatibility through transfer of accuracy to secondary reference materials and/or reference methods.

Secondary Reference Material

Secondary reference materials might also be pure substances or matrix materials. In the case of simple substances, purity should be assigned relative to a primary reference material. For a matrix material, values for analytes should be obtained by analysis using reference methods (when available) and/or be traceable to a primary reference material. The major use of secondary reference materials is for calibration purposes.

Control Reference Materials

Control materials may or may not have measured values assigned to them. They should be highly homogeneous and be used primarily for the purpose of estimating the random variability of the analytical process.

Development and Evaluation of Reference Materials

General criteria, which should be applied in the development and evaluation of reference materials are discussed in detail elsewhere.[5-8] Generalizations are difficult because each reference material presents a unique set of requirements with respect to such factors as accuracy, purity or stability. Identified below are some of the most important criteria which should be considered during the development of reference materials. It is essential to assess, for the particular type of reference material, the technical requirements that are dictated by the intended end-use. End-use will largely govern the specific analytical

requirements such as accuracy, stability, homogeneity, purity and form.

Pure Primary Reference Material

For pure substance primary reference materials, the following criteria should be taken into account:

a. The purity of the material shall be experimentally determined and stated along with an estimate of the uncertainty of the value.

b. Impurities shall be identified and quantitatively evaluated as required by the end-use.

c. The total uncertainty associated with the purity should be small enough that the material can be with a definitive and/or reference method without a significant effect on the overall measurement.

d. The homogeneity of the material shall be determined experimentally and any uncertainty due to material variability should be incorporated into the overall uncertainty.

e. The stability of the material should be established. Any criticial information concerning stability or special storage requirements must be provided.

Matrix Primary Reference Material

Considerations similar to those pertaining to pure primary reference materials also apply to matrix primary reference materials. Only well-defined, specific analytes in a matrix would be suitable for this classification. Matrix primary reference materials require the following technical points to be evaluated in addition to those cited for the pure materials.

a. Analytes must be determined by methods having the highest accuracy and precision. When available, definitive methods should be used.

b. Known or potential interfering substances present in the

matrix must be specified qualitatively and quantitatively.

c. The source of the matrix as well as processing procedures must be specified. In general, most matrix bases will be of a proteinaceous nature. When fractionation of serum has been performed to remove interfering substances, the procedure should be specified so that users may be advised of differences between the matrix and the starting material. Sterility considerations must also be taken into account.

Secondary Reference Materials

Secondary reference materials may also be either the pure or matrix type. The various accuracy levels of clinical need should govern the analytical specifications. Factors such as homogeneity and stability must also be evaluated and specified. The secondary reference material should be compared with a primary reference material, if available.

Control Reference Materials

Control reference materials should be evaluated for homogeneity. Depending on their specific use, they may or may not have measured values.

Establishment of Priorities, Specifications and Criteria

The establishment of general priorities for the development of reference materials and the specifications and criteria for specific reference materials should be carried out through the national consensus process as described above. In the past, NBS has used both experts panels (representing a broad spectrum of clinical community interests) and formal market surveys to obtain information needed to establish NBS clinical SRM production priorities. The National Clinical Chemistry Council and its various technical Subcommittees would function in this role in the future.

Validation of Reference Materials

The producers of reference materials must bear

Table 1

REFERENCE MATERIAL HIERARCHY

MATERIAL NAME	SYNONYM/ORGANIZATION	CHARACTERIZATION BY:	PROPERTY VALUE NAME	MAJOR USE
1. Primary Reference Material[a]				
a. Pure Primary Reference Material	Standard Reference Material (SRM)/NBS Reference Material/ ISO Primary Standard Material/IFCC	Definitive Method(s) and/or a number of independent methods of high accuracy	Certified Value	Assure measurement compatibility through transfer of accuracy to secondary reference materials and/or reference methods
b. Matrix Primary Reference Material	Standard Reference Material (SRM)/NBS Reference Material/ ISO	Same as 1.a. Homogeneity,stability, sterility, etc. considerations take on added importance	Certified Value	Same as 1.a.
2. Secondary Reference Material	Reference Material/ ISO Reference Material (Calibration)/NCCLS	Preferably reference method and/or comparison with primary reference material	Measured Value by a specified method (preferably reference method)	Calibration Standard; transfer accuracy to laboratory methods
3. Control Reference	Reference Material (Control)/NCCLS Control Material/ IFCC	Laboratory Method (if measured)	May or may not have measured values	Internal quality control

[a] These materials may or may not be certified by producer.

responsibility for assuring the reliability and accuracy of claims through reliable production and characterization practices. A formal validation mechanism would appear to be an extremely costly alternative.

DEFINITIVE AND REFERENCE METHODS

The term definitive method first appeared in a glossary for clinical chemistry among the provisional recommendations of the IFCC Committee on terminology.[9] The following is the definition that was given: "A method which after exhaustive investigation is found to have no known source of inaccuracy or ambiguity." Although not indicated, the new term was a replacement for "reference-line method" and "base-line reference method," the expressions used earlier[10] to refer to the isotope-dilution/mass-spectrometry (ID-MS) method for calcium[11] by which "target value" total calcium concentrations had been assigned to several serum pools. These serum pools were also analyzed in several laboratories by an atomic absorption spectrophotometric (AAS) method[12], and the results from the two methods were compared for the purpose of evaluating the AAS method as a reference method. It was because the accuracy of the ID-MS method was sufficiently high -- a more accurate method for calcium is not known -- that the comparison could be used for judging the accuracy-capability of the proposed reference method.

The IFCC definition cited above considers the degree of perfection of the definitive method, not the degree of accuracy needed for its intended use as a definitive method. Without the latter, the method would necessarily undergo only (possibly endless) testing for errors; whereas consideration of accuracy appropriate for its use builds in a condition which provides a practicable test to be fulfilled for recognition as a definite method. A revision of the provisional definition to include that essential characteristic is proposed:

> "A definitive method is an analytical method that is capable of providing highest accuracy among all methods for determining that analyte, and its accuracy must be adequate for its stated end-purposes."

Important end-purposes for the definitive method for an analyte are used in evaluating the accuracy of the reference method and use in determining definitive method values for matrix primary reference materials. It is because definitive methods are too expensive and inefficient to use for other standardization end-purposes (and are, therefore, reserved for primary analyses) that the more practical reference methods are essential.

In place of the IFCC provisional recommendation for the definition of a reference method [1], which reads as follows: "a method which after exhaustive investigation has been shown to have negligible inaccuracy in comparison with its imprecision," we propose an alternative that provides additional characteristics:

> "A reference method is an analytical method whose inaccuracy and imprecision are small enough, as demonstrated by direct comparison with the definitive method, and whose low incidence of susceptibility to known interferences is thoroughly documented that the stated end-purposes of the reference method may be achieved."

Some important reference method end-purposes are its use to evaluate the accuracy of other laboratory methods and use to determine reference method values for secondary reference materials. The setting of maximum inaccuracy and imprecision limits for a reference method to fulfill its end-purposes is the responsibility of the Council Subcommittee.

In a definitive method, systematic error is either absent or accurate corrections can be applied for the known systematic error; whereas in a reference method not all of the systematic error may be known and corrected or correctable, and hence it may exhibit some bias. Thus the definitive method should have greater accuracy than the reference method; however, an equivalent accuracy is also possible. Both methods must necessarily be capable of being performed with high precision.

The definition of a reference method imposes an

evaluation relative to a definitive method. Notwithstanding, situations exist wherein definitive method developments are not practicable: for example, the analyte is not a specific chemical substance (e.g., total protein); the analyte is defined, however an appropriate candidate method to serve as the basis for definitive method development may not be evident (e.g., bilirubin); the property under analytical consideration may be an activity rather than an analyte concentration (e.g., an enzymatic activity). Under such circumstances, the Subcommittee may wish to resort to naming a particular procedure as the designated reference method. However, as discussed below, a candidate designated reference method would undergo extensive testing before being accorded such status. If, after a designated reference method has been accepted, a definitive method is developed, an evaluation of accuracy would then be in order.

Definitive Methods

Definitive methods must be based on sound analytical procedures. For example, all the analyte in a sample is recovered as a pure substance (the amount of which is accurately measured) and all analytical residues are carefully tested for the presence of any unrecovered analyte, care being taken to avoid errors due to sampling, blank, etc. As another example, if less than quantitative recovery of analyte is involved, internal standardization is utilized. Quantitation is achieved from the relative proportions of isolated analyte and internal standard, each of which is determined specifically. Generally, the analyte as measured and the primary reference materials used for calibration are as alike as possible, and in both these examples, measurements are performed on analyte in as nearly pure condition as can be isolated. Where possible, use is made of instrumentation that enhances the specificity of the measurement, with the objective of minimizing, indeed absenting, interference effects from all sources.

The development of a definitive method might be undertaken in any laboratory (or in more than one) that has the resources, personnel and equipment. However, before starting, the laboratory should share its plans with the Council Subcommittee and then periodically report its progress. Since the definitive method must be capable of

being used to establish the accuracy of a reference method and only the minimum required accuracy and precision of the reference method are prespecified (by the Council Subcommittee), the performance aimed-for in a definitive method must be several-fold better than that.

In the use of the definitive method for evaluating the reference method, matrixed specimens are analyzed and pure primary reference materials serve for the calibration. If there are systematic discrepancies between the results of the definitive and reference methods, their cause should be investigated and, if possible, explained, since the consequence of the occurrence of differences is that reference method values would be adjusted to match the definitive method values.

Reference Methods

In selecting candidate reference methods (where a definitive method may or may not exist), several interrelated prerequisites must be satisfied. A candidate for the method should have a sound theoretical basis and have been well enough studied to show its potential for affording the accuracy and precision required as the reference method. Each method given consideration should be evaluated experimentally and statistically on a camparative basis to find the method with appropriate linearity, this choice of method, optimization of the experimental parameters for the performance of the candidate method should have been completed.

If the kind of instrumentation to be used for the measurement has a demonstrated stability over the total time required for analysis, then calibration of the entire analytical range before and after replicate sample analysis should be adequate to ensure good measurement. On the other hand, if the instrumentation is not stable (i.e., displays drift or noise that is large compared to the precision goals), bracketing of analyte concentrations with standards of similar concentration and interpolation of the data are recommended.

The written protocol for the reference method should be complete: this includes directions for the use of the

appropriate primary reference material, specifications for reagents and glassware, techniques for sample handling and solution preparation, and procedures for necessary testing of reagents and instrumentation. It should also include a section on recording data and examples of necessary calculations.

In the development of a reference method the final step is to demonstrate that the required accuracy and precision goals are transferable. The effective procedure for this demonstration is interlaboratory testing with a limited number of laboratories involved. A preliminary test that employs control materials as samples is run so that participating laboratories may become familiar with the protocol. This preliminary test and any subsequent initial interlaboratory testing should provide feedback on problems with the written protocol and opportunity to modify it if needed. Final interlaboratory tests are performed on serum samples for which definitive method measurements are also obtained. The testing by both methods is organized in close consultation with statisticians. The reference method results are compared statistically for within- and between-laboratory precision and, where a definitive method exists, testing is continued until it is determined that the accuracy and precision goals have been reached. If in the course of the study it is found that the accuracy and precision goals could not be attained by the candidate reference method, or are inappropriate, new goals could be set or some other change might be initiated by the Council Subcommittee; the clinical community would, of course, need to be informed of such a change. Where a definitive method does not exist, interlaboratory testing assures the transferability of the procedure and establishes the imprecision range that can be expected from the reference method.

Maintenance of Methods

A reference method is not intended for routine use nor for every laboratory, but may be used in laboratories with appropriate personnel and equipment. However, even for such a laboratory, in order to achieve the capability inherent in the reference method, its personnel must become familiar with the procedure and have it under quality control.

Since the special functions for a reference method are its use in determining reference method values for secondary reference materials and in evaluating laboratory methods, only laboratories engaged in such activities would need to maintain or otherwise have access to the method. They might include clinical research laboratories, suppliers of reference materials and of equipment and materials for laboratory methods, and laboratories in the U.S. might be expected to maintain reference methods.

A very few laboratories in the world may set up and maintain definitive methods. Several laboratories should be engaged in developing definitive methods simultaneously since so many definitive methods are needed. Also, there is the need to corroborate analyses obtained by definitive methods which, at the very least, would require a second laboratory to set up each definitive method. NBS expects to maintain the methods that are developed at NBS and to corroborate, if not also maintain, those developed elsewhere.

Some other aspects of maintenance need to be considered. First, changes in protocol. The protocol for a reference method completely specifies the method. If the protocol provides alternatives for a step in the procedure, for example, manual pipetting or mechanized pipetting, the specifications for those alternatives were tested during development of the reference method. However, if an alteration is introduced by an analyst to improve or expedite the performance of the method, that alteration must be considered to have so changed the protocol that the altered form is no longer recognizable as the reference method. While that alteration might effect the anticipated benefit, the fact must be demonstrated by an approved, multi-laboratory experimental study in order to be appropriately introduced as an acceptable alternative.

Second, permanence of these methods. The development of definitive and reference methods are major tasks. A considerable portion of the effort expended is to assure that the develoved methods are as correct as scientific capability mixed with practicality will allow. Practicality judgment has to enter the effort such that over the years the methods will serve as the measurement system requires. However, work done afterward may reveal shortcomings. The system should be

flexible to accommodate new findings and to make appropriate modifications when necessary.

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NATIONAL COMMITTEE

FOR

CLINICAL LABORATORY STANDARDS

POSITION PAPER

by

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INTRODUCTION

The NCCLS supports the concept of a National Reference System (NRS) for the Development of Reference Materials and Methods for Clinical Chemistry. The rationale for such a system developed in this paper is based upon the premise that any expenditure of resources in the development of standards must be directed toward the goal of improved patient care and must accelerate the development of new standards while continually updating existing ones in the clinical laboratory field. It is the position of NCCLS that such an accelerated development is desirable and practical, and can be best implemented through an existing voluntary consensus mechanism such as NCCLS. Such a voluntary consensus approach draws on the strengths of all elements of the laboratory community without imposing arbitrary or unilateral actions on any one group.

BACKGROUND OF NCCLS

In 1968, the NCCLS was chartered as a nonprofit corporation for the purpose of permitting the development of national standards for clinical laboratories, and providing a mechanism for defining and resolving problems that influence the quality of work performed. An important aspect of the new organization was that all of the contributors to clinical laboratory operation (professional, governmental agencies, and industry) were represented. The elected officers and directors who compose the NCCLS Board must, according to the bylaws, represent a careful balance of these three constituent disciplines.

Members of NCCLS may be private or public educational, scientific, professional, governmental, or industrial organizations. Each member organization is represented by one voting delegate and one alternate delegate. They are charged with the responsibility of circulating all documents and materials to appropriate individuals within their organizations for comment, promoting and supporting standards-development activities, and participating in the balloting for action on standards and election of officers and directors. The NCCLS membership currently consists of twenty (20) professional associations, seven (7) governmental agencies and seventy-two (72) industrial organizations, which

STANDARDS FLOW CHART

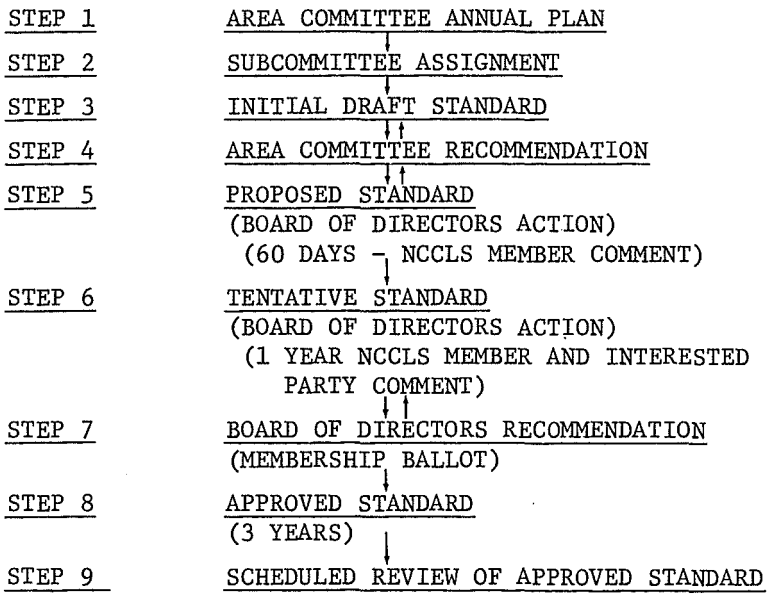

Fig. 1 MECHANISM FOR DEVELOPMENT AND ADOPTION OF CLINICAL LABORATORY STANDARDS BY NCCLS

cover the entire spectrum of clinical laboratory endeavor in the United States.

It is in the NCCLS committee structure that the research, development, writing, and revision of standards -- and thereby the goals of the organization -- are accomplished. At the present time there are nine broad Area Committees covering the major disciplines of the clinical laboratory. They are: Clinical Chemistry, Hematology, Immunohematology, Microbiology, Labeling, Instrumentation, Evaluation Protocols, Ligand Assay and Toxicology/Drug Monitoring. These Area Committees are chaired by recognized authorities in their respective fields. The Area Committees supervise the activities of numerous Subcommittees and Working Groups.

In addition, there are presently two ad hoc committees working on contract projects for the FDA: one on assessing the level of consensus on methodological principles for assigning principal assigned values to calibrators; the other on testing the applicability of a reference method for antimicrobial susceptibility testing of anaerobic bacteria.

As in all NCCLS activities, a careful balance of representation from all participating members is maintained on all committees. The close association of individuals with varied backgrounds in these working areas ensures that documents and specifications are broadly based.

Figure 1 outlines in schematic form the mechanism by which NCCLS develops, adopts, and reviews its standards. Standards may originate from a wide variety of sources. Typically, when the need for a standard is recognized, it is assigned by the appropriate Area Committee to an existing or newly-organized Subcommittee, Working Group, or Task Force. The Committee develops a draft standard, which is submitted to the Area Committee for review. The NCCLS Board of Directors then examines the Draft Standard, returns it for further study, if needed, or submits it as a Proposed Standard to the general membership for comments. The Subcommittee is required to answer all negative comment, after which the Board issues the document as a Tentative Standard. The Tentative Standard is widely circulated for one year during which time it is subjected to review,

comment, and challenge from the membership and concerned parties. The Tentative Standard may be returned for revision, or directly submitted to the membership for ballot approval. Approval in the membership ballot represents a consensus of those who are concerned with the scope, provisions, and use of the standard.

The NCCLS mechanism attempts to satisfy the conditions for consensus, i.e., substantial agreement (not unanimity) among the parties concerned. The time-consuming nature of standards development is directly attributable to this need for bringing multiple and divergent opinions to a common meeting ground.

In addition to committee preparation of standards, NCCLS may use existing documents, sponsored by individual professional associations, and move them through the consensus process. Government agency guidelines or regulations may be submitted to the membership to provide an opportunity for comment and feedback, with the aim of influencing revisions where the consensus process indicates a need.

While NCCLS concerns itself primarily with standards for use in the United States, it takes cognizance of the numerous standardization efforts internationally. Several such documents have been carried through the NCCLS consensus mechanism with important benefits to international cooperation in clinical laboratory practice.

NRS AND NCCLS GOALS ARE COMPATIBLE

The goals of the proposed National Reference System for the Development of Materials and Methods (NRS) in clinical chemistry appear to be essentially those of the NCCLS, i.e.:

a. To promote the development, evaluation, approval, and implementation of national and international standards for the clinical laboratory field in order to maintain national laboratory performance at levels consistent with the needs of patient care.

b. To provide a mechanism for approval of clinical laboratory standards based upon a consensus of all

parties interested in the scope, provisions, and use of such standards.

c. To cooperate with other national and international organizations in developing and approving clinical laboratory standards, promoting their use, and evaluating their effectiveness.

d. To maintain a forum for communication and mutual education among professional, governmental, and industrial organizations concerned with the quality of clinical laboratory performance and the relationship of standards thereto.

The real question then is whether or not the existing NCCLS voluntary mechanism can provide expeditious development of standards and efficient management of human and financial resources toward the goals of improved patient care. It is the position of NCCLS that it can. NCCLS believes that the existing, voluntary consensus approach to standards development offers the optimum solution to the proposed NRS in that standards generated within the system will impact directly on the quality of health care services and products in an age where health care has reached national policy proportions.

THE CHALLENGE TO THE NRS

The consensus standards approach is being challenged to make a positive contribution toward assuring the consuming public that they will receive safe, effective, progressive health care products and practices. Accordingly, if the proposed NRS is to be voluntary, it must establish procedures that are free from bias and open to critical review. The procedures must assure the best judgement of all segments of the laboratory community and must also be responsive to the need for modification and improvement on a timely basis.

If it is to be effective, the NRS must use a consensus approach in establishing priorities. Organizations and individuals will have to address the purpose, utility, and need for standards. Clearly, the major factor in the selection of priorities must be the contribution the standard can be expected to make to clinical diagnosis and laboratory

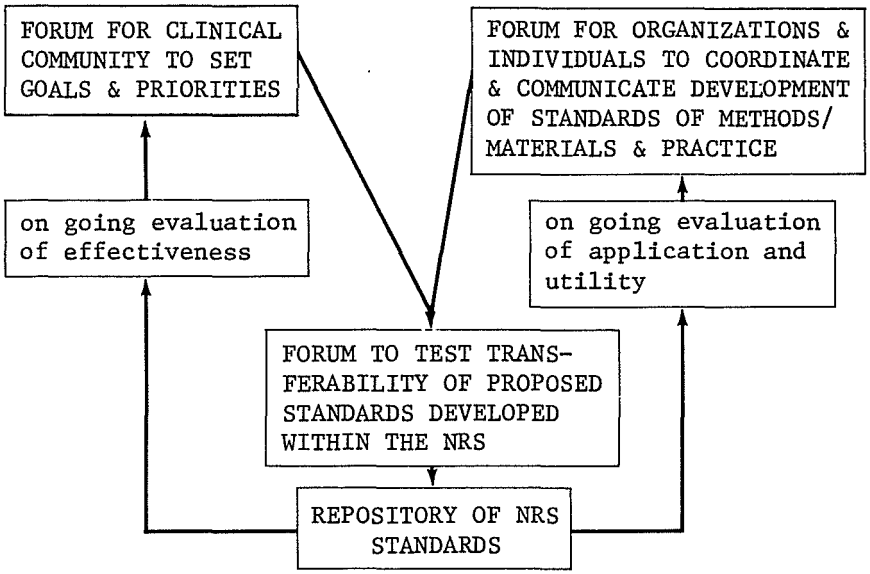
FORUM FOR CLINICAL COMMUNITY TO SET GOALS & PRIORITIES
FORUM FOR ORGANIZATIONS & INDIVIDUALS TO COORDINATE & COMMUNICATE DEVELOPMENT OF STANDARDS OF METHODS/ MATERIALS & PRACTICE
on going evaluation of effectiveness
on going evaluation of application and utility
FORUM TO TEST TRANS-FERABILITY OF PROPOSED STANDARDS DEVELOPED WITHIN THE NRS
REPOSITORY OF NRS STANDARDS

Fig. 2 NCCLS functions within the Proposed National Reference System.

performance. The process of identifying priorities for standard writing action may result in delay to subjects not selected for action, which may have had strong recommendation, but limited support. Priorities for standards will influence the level of participation that may be expected in the actual drafting, revision, and review effort. Inappropriate selection of priorities (e.g., a priority set to appease a special interest group) would be a waste of resources. It is the NCCLS position that there are two areas of standards compatibility with the goals of improved patient care. First, standards of practice; second, standards of methods and materials.

Our vision must reach beyond the straightforward concepts of quantitative measurements in clinical chemistry to include new and perhaps less quantitative fields such as immunochemistry, the biochemistry of microbes, protein chemistry, and the histo-chemistry of cellular identification. These fields reflect areas of great need in terms of patient care. Recent developments have shown that in many cases, the amount of analyte present is not as important as its character and/or its relationship to molecules, surfaces, and cells. Specific examples include characterization of enzymes in myocardial damage and location of proteins on normal and malignant cell surfaces.

THE NCCLS FUNCTION WITHIN THE NRS

Suggested NCCLS functions within the NRS are shown in Figure 2. NCCLS would provide an open forum to catalyze and conduct communication essential to identify individual needs, specific goals, and appropriate priorities for the NRS. Once needs and priorities are set, the development of standards would proceed through the appropriate member organization of the NRS. The Area Committee System of NCCLS would then be used to provide the window through which such standards pass and through which NRS participants view the progress and appropriateness of the consensus activity. Publication through the NCCLS would provide notice to all concerned about the activities of each group within the NRS. Once a standard has been developed by the appropriate group within the NRS, applicability evaluation and transferability testing could be processed through the NCCLS. Through this system of consensus review, the industry, government and professions

would have input into the properly developed and validated standards of methods and materials. Needed standards developed through this process would become functional within the NRS.

The NCCLS could further serve the NRS by becoming a national repository for such standards and a library of source information. Finally, the NCCLS could continually and dynamically evaluate the effectiveness and appropriateness of these standards to patient care.

This use of NCCLS is cost efficient because it uses resources that are already available to develop standards dictated by patient care needs.

NCCLS believes that the organizational component of the NRS might better be called the communication and consensus system embodied by NCCLS. The consensus communication resources of NCCLS would meet the primary NRS goal, through the definition of need and establishment of priorities relevant to the practice of medicine.

Standards of practice to ensure maximal clinical usefulness of the quantitative as well as the qualitative non-dimensional application of clinical chemistry should be developed through the various disciplines represented by the Area Committee System of NCCLS. Further, these standards could be subjected to the consensus process for transferability and application purposes. NCCLS would coordinate and manage the ongoing consensus review of approved standards to ensure continued effectiveness and relevance to patient care.

The NCCLS function and role within the NRS could be seen as open ended, catalyzing communication at the onset, while providing the consensus review mechanism through which all segments of the laboratory community could view and participate in the activities of the NRS. We propose that standards of practice and standards of practice and standards of materials and methods are inseparable; hence, the defined quality of purpose and role of the NCCLS within the proposed NRS.

SUMMARY OF RECOMMENDATIONS

Standards of practice and of methods and materials in clinical chemistry must be based on medical usefulness and clinical relevance. The development, review, and updating of such standards must be carried out through the process of peer review in a consensus environment.

Each organization and individual within the NRS must be encouraged to contribute to the standards development process based on ability and resources.

NCCLS should serve as the communication mechanism in the development of goals and priorities for the standard making process.

NCCLS should serve as the forum for member organizations of the NRS to coordinate the development of standards.

NCCLS should provide the initial and ongoing consensus review of NRS generated standards.

NCCLS should act as the repository of NRS standards.

CONFERENCE SESSIONS

November 16-17, 1977

Atlanta, Georgia

OPENING PLENARY SESSION
NOVEMBER 16, 1977
8:30 A.M.

DR. DAVID D. BAYSE: Welcome to Atlanta. We are honored to have with us this morning the new Director of the Center for Disease Control, and I use the word "new" a bit advisedly because Dr. William Foege has been with the Center for over ten years in various capacities - smallpox eradication, and assistant director for international affairs. He was chosen as Director of the Center fairly recently. It is a pleasure to introduce Dr. Foege.

DR. WILLIAM H. FOEGE: Thank you very much. It is indeed a pleasure to welcome a group that one of our scientists has called the "Who's Who of Clinical Chemistry." After a look at some of the materials that were prepared for this meeting and recognizing the years of experience and expertise that went into the preparation of these materials, I was struck by two things. Number one - how little I understand of what I was looking at. But, number two, what a historic occasion this is , indeed, and I use the word "historic" as one who savors history. You wonder if it would have ever been possible before this time to have had a group such as this combining a holistic approach and a voluntary approach to reach agreements.

It is obvious that we are all going to benefit from your efforts, those of us who are in the health field and those who will be benefiting from the health field. We do, indeed, appreciate the preparation that you have put into this meeting, your very presence here today and the kind of cooperation that has shown through in the preparation. To those of you who were here yesterday (at the NCCLS meeting), I don't have to repeat how important I feel clinical chemistry has been for CDC's principal goal, which is to prevent disease.

Many changes have taken place at the Center in the last 31 years that it has been in existence. During the 1940s we were dealing primarily with vector-borne diseases. In the 1950s we had challenges in infectious diseases, many of them now preventable by vaccine. In the 1960s we were dealing with some of the more sophisticated viruses, and viral

prevention is concerned with a variety of things; life styles, which can be as dangerous as the microorganisms have been; the environment, in which the technological development of our age has caused problems difficult to define and to solve. Our activities at the Center have reflected the changes of the last 31 years and primary to this total picture, of course, has been the development of clinical chemistry. Basically, the Center has responsibilities to improve both health care and preventive medical services to the American people by working through laboratories in two ways: First, we offer support to Public Health Laboratories at the State and local levels. We try to foresee problems as well as react to Public Health emergencies and problems as they develop. But second - we feel that we are responsible for improvement of health care delivery to individuals by ensuring that clinical laboratory services are of the highest quality and dependability. For total effectiveness, laboratories must have a uniformly high quality of performance which is standardized at a level that will give all physicians and all patients comparable levels of reliable service. I have the feeling that social justice is in fact the philosophical base of public health, and one facet of social justice is that people have equity of access to medical care services including laboratory services. It has become apparent that adequate support of the physician as he applies laboratory information to the management of his patients requires greater standardization than we have had in the past. I would like to provide a few examples of what CDC has been doing in this area and first start with some domestic examples.

We have for some years been supporting the National Center for Health statistics in their national survey of the health status of American people. The last 8 years this has concentrated heavily on nutritional status. In addition to assisting in the field examinations, we have provided the central laboratory backup for these services and we are working at this time particularly on developing a clear definition of the nutritional base and prevalence of anemias. In the chronic disease area our laboratory bio-chemistry programs have long concentrated on cardiovascular diseases in cooperation with the National Heart, Lung and Blood Institute. Much of the laboratory standardization effort is devoted to key epidemiologic and intervention studies of

large scope, such as diet and drug intervention programs. And with increasing frequency, CDC epidemiologic investigations involve illness related to chemical toxicity in high-risk populations and in outbreaks of exposure to chemicals of industrial, agriculture or other origin. At the present time, we are devoting much effort to the problem of lead poisoning both in epidemiologic measurements of exposure and in the administration of the National Childhood Lead Poisoning Program.

We have, of course, for the past ten years had legislative responsibility to assist the nation's clincical and public health laboratories to reach consensus goals on medically useful levels of reliability. Our programs are designed, whenever possible, to work through professional organizations and through state programs. Programs with statutory responsibilities for assessing proficiency of clinical laboratories also have, we feel, a responsibility for assisting those labs to achieve greater proficiency. One way to attack this problem is by seeing that laboratories have effective and consistent tools with which to work. We have, of course, been working with the Food and Drug Administration's Diagnostic Product Regulatory Program in this effort. If there is one reality that has become clear to me in this nuclear and space age it is that the future requires that we be not only American citizens (nationalists), but that we indeed be global citizens. We can never define national boundaries as the ultimate horizon of our responsibility in providing good health. Decisions which will ultimately serve national interests best are those which improve world stability and move us all toward a genuine world order based on health, knowledge and productivity.

CDC has in its past history had a strong orientation towards international activities and we continue to see--to hope--that our future will be one oriented towards international activities. What are we doing in the international field? I will mention just a few examples: Through the Pan American Health Organization and through WHO we have supported epidemiologic and intervention studies to produce comparative health data among defined populations in other parts of the world. Most of these studies have concerned cardiovascular disease and diabetes. We have just

completed a large study of the prevalance of hyperlipidemias arranged by international agreement between the U.S. and the USSR. The need for international cooperation in some other health problems has brought into sharp focus the problems of chemical toxicity. These usually require prompt mobilization of technology to bring serious outbreaks under control, and we have cooperated with a number of countries and international organizations in this regard. The very serious exposure of the residents of Michigan to PBB has been paralleled, as you know, by a similar situation in Italy in which an explosion in a plant manufacturing trichloriphenols exposed a heavily populated area to extremely toxic dioxans. Cases of excessive exposure of infants to hexachlorophene in this country are matched by similar experiences in France where some forty infants died because of much heavier exposure. In both instances CDC has been involved in sharing its experiences and its technological capability to define the degree and extent of exposure. Examples such as these, point to the urgency for standardized laboratory support of public health and individual medical management on a world-wide scale.

Clinical chemistry has made dramatic progress through the years, much of it because of people sitting in this room. Progress in the future will depend on the work that you will be doing here in the next two days specifically, but also in the years to follow. A National Reference System for Clinical Chemistry which I think will in fact become an international reference system for clinical chemistry, will be one of the great contributions you can make to those who come after you in this field. Again, I would like to thank you for the contributions you have already made preparing for this Conference, and for the contributions that you will make in the next two days. I wish you outstanding success.

DR. BAYSE: Thank you, Dr. Foege. Dr. Foege has mentioned the potential international implications of our deliberations. We have invited a few very key international people to come as observers to avoid some of the complications of a purely localized solution by suggesting some future international cooperation. I would like to welcome Dr. Stanley Brown who officially represents IFCC; Dr. Jim Logan of the CDC of Canada; and Dr. Koedum of the Public Health Laboratories of the Netherlands; Professor Jean

Pierre Bretaudiere represents the French Biological Society; Professor Dankwart Stamm of the Max-Planck Institute represents the German Association of Clinical Chemists and Dr. Wolfgang Gruber from Boehringer-Mannheim in Germany. We are very glad to have you as our guests and will hope that your observations and contributions will be fruitful to our future cooperation.

All of the sessions will be assisted by the clerical staff of CDC in which each of the sessions will have a secretary from the Center accompanied by one of our scientific people to assist.

We come here today to discuss a topic which I think could be summarized as the basis of measurement in the clinical laboratory. We have chosen the topic of clinical chemistry because of all the disciplines, it has advanced further on a quantitative basis and the people that are involved with it are, I think, better able to plan for its very quantitative future. The future of this development as it has been described in the Conference brochure is as a national understanding concerning the development of reference methods and materials. We should not be concerned that we are discussing only a narrow aspect of the national measurement system. The title was carefully chosen. An Understanding is not necessarily change. Change must be fashioned out of demonstrated need in the form of unmet past goals or future challenges. Before we talk of the future which will be done later in your working group sessions, let me review the past a bit.

I would like to look at the accomplishments and the expertise that have combined to make this conference possible because I would like to emphasize that there is a system in place. First, beginning with the NCCLS, we have an organization that has one of the broadest bases nationally in clinical chemistry. It embraces not only government, professional societies, but also industry--the in vitro diagnostics industry. And it has had, I think in the last two or three years, two very significant accomplishments in clinical chemistry. One is a standard for calibrators and control materials and the second is a proposal for methodological reference principles. I think both of these have been very unique contributions.

The National Bureau of Standards has made outstanding contributions in the area of definitive methods and in SRMs and has shown a great deal of leadership in developing ideas concerning the basis of measurement in clinical chemistry. The AACC has had an outstanding record, particularly from the standards committee that has developed many proposed reference methods in the area of glucose, uric acid, total protein and in also recently instrumentation guidelines.

The FDA has, I think, done a national service in their published labelling regulations and has over time sought broad based scientific input across the whole sector of the medical community.

The industrial group in the in vitro diagnostic area has made available to the medical community an array of products that only a few years ago would have been considered impossible: automation and micro-techniques are standard items at a relatively low cost and that would have been an impossibility only a few years ago. Additionally, this particular group of people has shown a lot of professional leadership.

The College of American Pathologists very early developed what has become an invaluable national resource in its survey or proficiency testing. This program is the largest of its type in the world and has had many features that have made it a unique part of our national reference system already. The CAP also has programs in the area of product evaluation that have rendered important national service.

The ASCP has sought for a long time to upgrade the profession especially through education and now regularly has a number of national meetings every year in its drive to bring a greater awareness and understanding of the profession of clinical medicine and therefore to improve it.

As you well know, at the Center for Disease Control we put, as Dr. Foege says, a lot of effort in the area of clinical trials and standardization and have developed recently a number of reference methods and materials.

All of this points out that we do not come to this room

empty-handed, but with a lot of talent and concrete accomplishment. We also come to this room with an existing system. The main characteristic of this system is that it does lie within individual organizations and is not, in an all over way, coordinated. We today must consider whether a further coordination and development of these programs is warranted. In this audience there are very divergent feelings on that subject. Therefore, this Conference is convened to afford an opportunity to examine the development of a National Reference System and whether or not further changes are needed to meet our future challenges.

For my final remarks, I would like to share with you a piece of literature that I have always enjoyed written by Lewis Carroll. He was a very advanced person for his time. He has a unique place in literature. I would like to share with you "The Lobster Quadrille," in which Alice, after wandering through Wonderland comes upon two rather ridiculous characters, one of which is a Mock Turtle and the other is a Griffin which the passage describes by saying "see the picture." Meaning that you cannot describe this ridiculous animal; it's a large bird that has wings but doesn't look like it would ever get off the ground. Some might think that is a caricature of a civil servant. Any way, after some coaxing, Alice gets the Mock Turtle to share some of his experiences with her; he reveals that he has a compulsion to organize things. He therefore recites the problem in verse; he desires to organize a group of lobsters that he finds on the beach and after proposing the organization he then holds out the delights the organization will bring and finally trys to allay their fears because part of this quadrill consists of being thrown out to sea and the lobsters are getting a little frightened over the fact that they may be thrown too far.

I would like to share a little bit with you in what follows because I think it is germane to this meeing. "Will you walk a little faster," said the whiting to the snail, "There's a porpoise close behind me and he's treading on my tail." Then it goes - "How eagerly the lobsters and the turtles all advance; Will you, won't you; Will you, won't you, come and join the dance?" And then in the area of delights, "You really have no notion how delightful it will be when they take us and throw us with the lobsters out to

sea." And then the allayment of fear: "What matters how far we go?", his scaley friend replied, "The furtherest from England, the nearest it is to France."

After finishing these some 18 lines, we find that Alice said "You don't mean 'porpoise'- you mean purpose" and also there is some discussion over whether whiting is something one serves at a dinner table or puts on one's shoe. Therefore, all three go off not understanding what they hear because they have a problem called "definition of terms."

Next on the program we have neither a Mock turtle, a Griffin, nor an Alice running aimlessly through life.

On the program next, we have a scientist, and a national leader in Clinical Chemistry. Dr. Ralph Thiers will deliver the keynote address on the experiences of a clinical laboratorian and his frustrations in the need for points of reference in clinical chemistry.

We appreciate Dr. Thiers coming down this morning. I've learned this morning that he has been somewhat ill and we do appreciate his special effort. Dr. Thiers.

DR. RALPH E. THIERS (Bio-Science Laboratories, Van Nuys, California).

Ladies and Gentlemen:

It is a real privilege, and a deep responsibility for me to present the keynote address for the Conference for an Understanding of a National Reference System for Clinical Chemistry. If an understanding can be reached at this Conference it will be an accomplishment of which every one of you should be very proud. It will be a difficult accomplishment, because the groups you represent diverge widely in their interests, backgrounds and goals. Careful preparation has been given to the positions each group plans to take here. Serious discrepancies of opinion and conviction exist and need to be resolved if the Conference is to succeed. But each group represented here and each individual present here has one interest in common and one underlying motivation--service to human beings at a time when they need it most, when they are ill. I believe this overriding common purpose will make this Conference succeed.

Since I accepted the invitation to give the keynote address my telephone has been ringing with unusual frequency. Many of those in this audience called to express their hopes and fears for this Conference. Fears outnumbered hopes by a wide margin. Because of this, I plan today to divide my talk into two parts. The first will comment on our present status with respect to reference methods and materials in clinical chemistry. The second will list, in frank and blunt fashion, the classes of fears which have been related to me with, at no extra charge, my own personal advice concerning each of them.

I'll never forget when I first started directing a clinical chemistry laboratory and it came time to look at the bilirubin determination. The morning that I observed the performance of that particular determination I noticed the technologist take the rack of tubes of serum that were to be measured, pick out the first one on which a bilirubin had been requested, look at it and place it right back in the rack. However the second one she looked at she placed in the rack for a bilirubin-determination. The third one went back in the storage rack, the fourth one also went back in the storage rack. The fifth one, in the bilirubin rack and so on. I asked her what she was doing, and she said, "Well, those are all within the normal range, below 0.6 milligrams percent." (We used milligrams percent in those days!) And I said, "Can you really measure that by eye?" She said, "Sure." And I said, "Are you sure it isn't 0.7 or 0.8?" "Well," she said, "it may be up to 0.9, but that doesn't matter very much." "Well," I said, "What about 1 or 1.1?" "Oh, definitely, I can tell that difference," she replied. "Well," said I, "you surely are giving the patient a break in that there is no charge for that quick look." "But there is," she said, "they get charged the same $5.00 as those over in that rack." I checked this with the physicians and to my surprise this system made perfectly good sense to them. They were interested in the bilirubin determination, they said, only as a confirmation of their visual observation of jaundice. They were using a quantitative measurement purely qualitatively.

But other things proved to be wrong with the bilirubin determination. I observed that you just didn't see values much above 14 or 15 in that laboratory. I knew there ought

to be a few above that now and then so I questioned the calibration procedure. I looked at the bottle of so-called crystalline bilirubin which they were using to make calibrators and was shocked to find that looking at the bottle I could see three different hues of brown. I ordered a new bottle of bilirubin and got a fourth hue. Which one was the true bilirubin or were they all good? In those days, who knew? That was years ago. But the situation never even began to get cleared up until the first commercial preparation came along which included on the label a high known value for bilirubin absorbance. But look where we stand today on that problem. We have excellent standard reference material for bilirubin. We have new methods for bilirubin. And bilirubin analysis today is not used simply as a check on visual observations of jaundice. It is used in a much more sophisticated and quantitative fashion. We've come a long way.

Another example that sticks very much in my memory involves calcium and illustrates accuracy, precision, and problems with procedural writeups. Again, this occurred years ago - at a time when there was only one commercial preparation which could be used as a control for calcium, and even it was new. Our laboratory was using that reference material. I was quite proud of our calcium method, and the quality control charts always looked very good. But one day clinicians suddenly began complaining that our calciums were low. Well, as all of you know, if the clinicians don't complain about a test that doesn't mean it is good. But if they do complain about the results, you had better look at that procedure no matter how good you think it is. However, for the first couple of days, I simply referred the physicians to the chart on the wall. The accuracy was as beautiful as ever and the precision which had always been good had improved markedly quite recently. But they still complained, so I investigated. I went through it in the usual order that has proved the fastest way to trouble-shoot, starting off always with calibrators, looking to see if there is anything wrong there, then controls, then reagents, then instruments, and, finally, people.

In this case I didn't get beyond number one and two. It turned out that one of the technologists in her superior wisdom had decided that this control material, with a known

value of calcium, would make an excellent calibrator. The procedural write-up called for its use as a control at various blind dilutions. She had bootlegged it into the method as a calibrator. That wasn't the way the procedure was written. But she did it and it worked very well for a while until, unfortunately, at the company which made that particular control material, some technician changed their procedure without notice and they managed to put out a batch which was incorrectly labeled. The stated concentration was erroneously low. So there we were in blissful ignorance! We had a beautiful control chart, with precision looking better than ever, and we were reporting serum calcium values, all of which (100%) were incorrect!

I can't resist telling you the story this always reminds me of. It comes from E. Bright Wilson's book, "An Introduction to Scientific Research" and it concerns a Swiss Bell Ringer. He had the job of ringing the bell at noon every day in a little Swiss town. He was very proud of his watch. He went into the jeweler in the town one day and he said "This is a superb watch. It belonged to my father and grandfather before me, and it keeps perfect time. Every day before I go to ring the bell at noon, I check it against your big chronometer on the wall there. In the last twenty years I've never had to set it more than a few seconds." The jeweler said, "Well, now I can tell you why. I always set the chronometer by the noon bell." Now, you'd think that this problem would be such an old story as to be unimportant. But, in fact, the last time I had to argue with someone that an illegitimate relationship between calibrators and controls can cause problems was only six months ago. And over the years I've had this argument frequently, and not only with microbiologists. It is a fight that never seems to cease.

This story illustrates another point which, to me, is the most frustrating of my entire analytical and clinical chemical career. That is, that you can write a procedure so that any fool can understand it and so that nobody could possibly go wrong in following it, but you have no guarantee that the method will be run by that procedure even by the best intentioned worker. Or, to put it as bluntly as I can - I have learned the hard way, that regulation by written procedure is totally ineffective. I suppose we ought to know that. They don't regulate us very well to drive under 55 mph

by writing clear-cut rules stating that we should. The only way I have found to check quality of product is by checking the product itself, not by trying to dictate how the product is to be made. That last sentence bears frequent repetition. People do not do what is expected. They do what is inspected.

Today the number of control materials available for calcium and other common analytes leaves no excuse for any laboratory to fall into the Swiss Bell Ringer trap. Inspection of performance by means of a variety of materials is possible and advisable. This Conference hopes to begin to systematize the field.

Let me go on to the other part of what I wanted to discuss - the fears you in the audience have for this Conference. The list is nine fears long. In each case I will pass on to you the fact or the fear communicated to me, then my personal reaction about what we at this meeting ought to do about it.

First, some of you fear the apparent assumption that a national reference system for clinical chemistry as a single, closely-integrated structure, is (a) possible and (b) good. So all of you might bear in mind that this is an assumption. I don't think anyone is trying to slip it past you as a tacit assumption without having you test it. It's your job at this meeting to try that assumption. Is such a structure possible? Intellectually? Mechanically? Are materials and methods inseparable? These are problems that you have to wrestle with.

Second. Many fear that this conference is trying to fashion a tool for an undefined task. Any task must be extremely carefully defined before the tool to solve it is designed. You can design the finest tool in the world, with the sharpest cutting edge, but unless you have defined the task you are going to use it for, it is going to cut away at thin air, much to your frustration, or worse than that, cut the wrong thing, which can be disaster. Let me expand on this by an example. The goal for this Conference stated in the papers you have in your hands is "to further the understanding of the components necessary for the development, the acceptance and the utilization of reference

materials and methods in clinical chemistry." That is a fine sounding statement. But it needs some definition all the way along the line. For example, take several words about the middle of it. "Development." Development by whom? "Acceptance." What do we mean by acceptance? Who is going to do the accepting? "Utilization." Who is to do the utilizing? How is the utilizing going to be done? "Reference materials." "Acceptance of reference materials." Is there a problem? Are they not coming out fast enough? Are the priorities wrong? The same questions apply to reference methods. Where does the problem lie? If we are going to design this system as a tool, let's get the problem it is going to solve very, very clearly defined. Again, my maxim is, define the problem before you design the tool.

Third. Some fear that the national reference system in clinical chemistry is to be a regulatory tool. The antidote here is to discuss this fear openly. Is regulation needed? Is this system (or part of it), appropriate for regulation. If you come to the conclusion that parts of this system should be used for regulation, design them that way and if you think parts of it should not be, design them so they cannot be.

Fourth. Forced acceptance of reference methods developed by some prestigious group and their use in regulation is a real fear. It is indeed important that no group be allowed to dominate the field of reference methods. It is very, very important to give reference methods thorough peer review, and to take them slowly. What works well in one laboratory may not in another. A good reference method must have transferability as a key property.

Fifth. Heedless replacement of present structures may occur. My suggestion here is to examine the performance of existing facilities and support them if they are performing. It may be tempting to say "such and such an organization is doing such and such a job, but they are too slow." If you replace that organization, their successor will be surprised by some problems that their predecessor has already run into and is working on, and the new organization will probably be at least equally slow. Support and enhance the existing facilities, then if this fails replace them carefully.

Sixth and Seventh. At this meeting (a) priorities,and (b) cost-benefits would not be given enough thought. These fears came up repeatedly. Advice on these is easy. That's your job - think about them. Make your colleagues here understand the priorities problem and the cost-benefits as you see them.

Eighth. An inhomogeneous group such as this one is going to have extreme trouble working as a unit. First, there is the government group. It has a clear mandate for the meeting. Then, there is the industry group. It has no mandate for this meeting. Each professional association group can take a definite position and has. And the academic people, of course, are, in theory, as free as a bird. That's quite a variety of goals and viewpoints to hope to unify. How can we hope, the fear runs, to make any kind of a decision.

But it has been shown that it is possible to make decisions even with that wide a variety of people. The process involves and implies finding a "consensus." My simple definition for consensus is (there are much longer ones) "if you can get everybody to go along with a given idea to the extent that no one in the group says "I cannot possibly abide that" - then you've got a consensus. Such decisions work extremely well if everyone has had a chance to be heard.

Ninth. Finally, there were strong fears expressed that the vested interests here represented may turn this meeting to their own profit--or even abort it to their own profit. Government vs. industry, for example. Professional associations vs. government. Those associations vs. industry. Ph.D. vs. M.D. All of our old "versusses." Clinical chemistry vs. Microbiology and each of those vs. Hematology. This is not to be feared. We have all got vested interests. They are not necessarily bad. The triumph of this meeting is that we are all here together with our vested interests. And we are all united by a strong common bond. We are interested in what's good for the patient. So, let's get to work then and use these vested interests. Let us decide what needs building here and get to work building it. But let me assure you that even if it turns out that you decide that nothing needs building then you have constructed

an increased understanding between the various people and groups here. That all by itself, is important enough to justify this meeting.

Thanks very much.

DR. BAYSE: Thank you, Dr. Thiers. Each of you in the agenda will find your session and your identification of the room and the observers. All meetings rooms are immediately under this room and you take the escalators down to the next level. The rules of the day will be discussed with you at that time, which were written by Jerry Gallwas, and if there is any question, you should discuss it with your coordinator.

Thank you.

The meeting is adjourned.

WORKING GROUP SESSIONS

OPENING REMARKS

The topic coordinators opened the joint topic sessions by outlining the purpose and procedures of the Conference. Mr. Gallwas opening remarks are summarized. Similar statements were made by Dr. Eloise Eavenson for Topic 1, and Dr. Thorne Butler for Topic 2.

The purpose of this joint session is to set the stage for individual working group discussions. Many of you have come with great apprehension, considering this a meeting of crisis. Many others come with great hopes and consider this meeting to be an opportunity.

I suggest to you that the only crisis will be if you, as participants and observers, do not exercise this opportunity to contribute to these proceedings. The Conference is structured as a meeting to discuss organizational views. Organizations, however, are people and people respond to logic. Therefore, your first charge is to persuasively sell your ideas and those of your organization to the Conference. Do not retreat as a disillusioned youth. Rather, take on this enterprise with the energy of a young reformer and challenge existing customs, current practices, (and your colleagues for that matter).

I caution you not to grasp at attractive short-range solutions to problems with long-range consequences. Certainly, you should not seek change purely for the sake of change without some reliable index of its ramifications. As you debate the issues before this Conference, remember that the goal is an understanding of the issues and of the possible alternatives to, what is in fact, the current system. The goal is not tranquility.

Within each topic area, there are significant differences of view. Therefore, as we go into the working sessions, the first order of business will be for each participant to restate the position of his or her organization. This will not only provide the organizational participants an opportunity to refresh their understanding, but will also serve to provide the observers a basis for the

subsequent discussion. Following these brief introductory organizational statements, there will be a 30-minute period for the organizational participants to question the views expressed in the opening presentations. This discussion of views may spill over into the working lunch, but should provide the basic information to set the tone and direction for group activity. Following this discussion, the working group chairman will address the issue of the agenda which will be reviewed and modified in accordance with the needs of the group.

It is important in these discussions that each group operate with guidelines understood by all; therefore, I will read to you the guidelines that have been discussed by all of the organizational coordinators, the group chairman, and which have been agreed to by the Chairman of the Conference, Dr. Bayse, as well as the Topic Coordinators, Dr. Butler, Dr. Eavenson, and myself.

PROCEDURAL GUIDELINES

The overall purpose of this Conference is to take the initial significant steps to explore an understanding about the organizational, material, and methodological elements of a National Reference System in Clinical Chemistry. To this end, the Conference organizers have solicited the views and recommendations of the participating organizations. We anticipate these submissions, already have and will continue to encourage a dialogue among those of you representing those organizations, as well as among those of you who are observers.

In order for the working groups to conduct their business, it is appropriate for them to summarize their deliberations in the form of recommendations with underlying reasons therefore. While it is not intended to submit working group recommendations to the overall Conference for approval, these recommendations and underlying reasons will be forwarded to the participating organizations and others in the laboratory communtiy within two weeks after the Conference for further review and discussion; and they will become part of the Conference Proceedings.

In order to facilitate accomplishing these objectives,

the following guidelines will be used:

1. Each working group chairman will summarize at the end of each discussion on a particular topic, the essential nature of the points discussed, as well as any agreements reached and any dissenting views. It is understood that there may be topics addressed where no conclusions can be reached, nor recommendations made.

2. In order to assess the level of agreement on each point of discussion, the working group chairman may call for a vote of the participants after having explained the effect of an affirmative or negative vote. Each participant's vote will be recorded as "For," "Against," or "abstaining." It is understood that the vote represents the participant's best judgment as to the view of his particular constituency, but in no way binds his or her organization. The chairman will be the only participant in the working session who can call for a vote on a point of discussion.

3. Individual observers are expected to contribute either in the form of written recommendations (which may be subject to a vote) or verbally in discussions. Appropriate times designated by the chairman of the working group sessions will be set aside for this purpose. Observers must be recognized by the chairman in order to address the working groups within the time limit designated.

4. Written comments from participants, their sponsoring organizations, individual observers and other interested parties will be accepted for a period of 60 days after the completion of the Conference. These comments must be stated succinctly and signed. They will be subjected to editorial review and then become part of the Conference Proceedings.

WORKING GROUP SESSIONS

TOPIC 1. Organizational Development of a National Reference System for Clinical Chemistry (NRSCC)

Topic Coordinator: Dr. Eloise Eavenson (FDA)
Orientation, Procedural Instructions, and Guidelines

Joint Session

Dr. Eloise Eavenson presented the rules under which the Conference was to proceed. Brief discussion followed, and Dr. Boutwell replied to a question by Dr. Roy N. Barnett regarding the editorial policy for the published proceedings.

GROUP 1.1 Analytical Goals and Quality Requirements

Chairman: Dr. Franklin Elevitch (CAP)

Participants:
Dr. Alan Mather (CDC)
Mr. Henry L. Lee, Jr.(FDA)
Dr. Philip D. LaFleur (NBS)
Dr. Robert W. Pritchard (NCCLS)
Dr. George N. Bowers, Jr. (AACC)
Dr. Desmond Burke (ASCP)
Dr. Roy N. Barnett (CAP)
Dr. Stanley Bauer (Industry)

Observers:
Ms. Marylin Amrik (American Monitor)
Dr. Joe Boone (CDC)
Dr. Theodore Carski (Becton-Dickinson)
Dr. Walter Frajola (AACC)
Dr. Spencer Free (Smith Kline)
Dr. Arthur Gottman (Coulter (SAMA))
Dr. Maurice Greene (FDA)
Dr. Eugene Harris (FDA)
Dr. Allan Hershman (Harleco)
Mr. Nick Kauffman (FAMA)
Dr. Ernest Maclin (Electro-Nucleonics)
Dr. John Mandel (NBS)
Dr. Horace Martin (AACC)
Dr. Robert Paule (NBS)

Dr. John Ross (CAP)
Dr. A. V. Roy (DOW)
Dr. Robert Taylor (DuPont)
Dr. Norbert Tietz (AACC)
Dr. O. W. vanAssendelft (CDC)
Mr. Richard Webb (Warner-Lambert)
Dr. Anne Yobs (CDC)

Assignment:

To attempt to identify those laboratory applications requiring specific performance levels. To evolve a framework for decision making that can serve as a basis for defining such goals in terms of medical usefulness in the development of reference methods and materials. Such a framework could provide a bridge between routine clinical laboratory practice and a national reference system.

Initial Agenda

1. Position Paper Summary Statements by each of the Participants

2. Participant Discussion - Definitions and Topics

National Reference System	Analytical Specificity
Reference Method(s)	Analytical Sensitivity
Reference Material(s)	Analytical Accuracy
Definitive Method	Analytical Precision
Field (Routine) Method	Clinical Sensitivity
Method/Materials Relationships	Clinical Specificity
Analyte	

3. Discussion Questions

   A. What factors provide a natural limit to the requirements for analytical sensitivity? Analytical Specificity? (Discuss population or group variability, individual variability; screening requirements; serial testing.)

   B. How can the influence of preanalytical factors be related to these considerations? (Discuss specimen collection, diet, stress, storage, transport, etc.).

C. Can knowledge of group and individual variability be used to set analytical goals for precision? Accuracy?

D. Can a formula for these goals be developed?

E. Assuming a consensus as to the goals for the analytical parameters of precision and accuracy (as well as clinical sensitivity and specificity), for methods in routine use or to supply patient information to physicians, then:

(1) What is the relationship between the analytical parameters of field methods to those of reference methods?

(2) Similarly, what is the relationship between the desirable analytical parameters of reference methods and definitive methods?

(3) Similarly, what is the relationship between the desirable characteristics of routine calibration and control materials used-

(a) In field methods?

(b) In reference methods?

(c) In definitive methods?

REPORT OF WORKING GROUP 1.1

The Group met in two sessions on November 16 and 17, 1977. The first sessions began with eight concise presentations of those portions of the position papers which were relevant to setting analytical goals and quality requirements. Each presentation was followed by 15 minutes of discussion. The day concluded with an exhaustive general discussion which covered a wide range of topics, including:

Precision
Accuracy
Medical relevance
Performance requirements

Product regulation
Patient risks
Economic costs
Preanalytic variables
Analytic variables
Biological variability
Matrix effects
Sensitivity: medical and chemical
Specificity: medical and chemical
Transferability of accuracy
Clinical use of laboratory data

During the evening of November 16, the group chairman prepared eight draft recommendations based on the day's discussions.

During the second session on November 17, the eight draft recommendations were revised by the group. Each was finally accepted by consensus. These recommendations form the body of this working group report. In order to illustrate the types of issues which were considered, each recommendation is followed by an editorial comment. While the eight recommendations were accepted by the group prior to adjournment, the editorial comments reflect the Chairman's best recollections of what transpired as critiqued by participants during the 60-day review period following the session.

Substantial agreement was reached on goals for short- and long-term intralaboratory analytic variability. On the other hand, much work remains in goal setting for long-term interlaboratory analytical variability, as well as understanding the relative contributions of random variability, constant bias and random bias to goal setting.

CONFERENCE RECOMMENDATIONS

The primary mission of a National Reference System for Clinical Chemistry is to provide a framework within which true values in clinical chemistry can be sought in order to achieve broad interlaboratory compatability over long periods of time. This system must be designed to promote individual and group initiative, responsibility, creativity and cooperation in applying the highest scientific principles to

patient needs.

A. Performance specifications for a clinical analytical method should be defined in terms of precision and accuracy and the major factors affecting them (such as matrix effects and the effects of interfering substances) at specified levels of concentration or activity.

Comment

Both precision and accuracy must be used to characterize all types of clinical analytic procedures, ranging from definitive to field methods. Accuracy is defined as closeness to the true value of an analyte in a matrix. Determination of the true value is based on the best available technology. Precision is commonly defined in terms of reproducibility in order to consider analytic limits of detection and the performance of the method at different levels of an analyte. There are varying amounts of data available to describe the matrix effects and the effects of interfering substances, particularly drugs, on the determination of analytes. The effects of these factors may vary at specified levels of concentration or activity of an analyte. Intermethod and interlaboratory biases of analytical measurement should be related to the true value of the analyte.

B. Models should be developed which link the performance of analytical methods to medical needs.

The state of the art in most instances permits setting analytical precision goals to less than one-half of the biological variability of the analyte as defined in the Conference Report and Recommendations of the 1976 Aspen Conference.

Comment

Laboratory results should be meaningful in terms of a clinician's ability to use this information effectively in patient care. One use of analytical results is to distinguish between patients with known diseases and those considered to be free of disease. In addition, prevalences of diseases also must be known. The data base may be developed through studies which utilize such contemporary hospital processes as medical audits.

Standard diagnostic nomenclature should be used, i.e., SNOMED. The use of multidisciplinary expertise (particularly clinical), information theory, and statistical models will be required to quantify the interrelationship of analytical performance and medical needs. Present knowledge permits us to relate analytical precision (coefficient of variation) goals to biological variability as recommended in the 1976 Aspen Conference.

The Aspen Conference recommendations for analytical precision are:

Biological variability is made up of intraindividual and interindividual components.

For group screening:

$$CV_{anal} \leq \sqrt{\overline{CV}^2_{intra} + \overline{CV}^2_{inter}}$$

and for individual testing:

$$CV_{anal} \leq 1/2\ CV_{intra}$$

the rationale being that if the analytical uncertainty is less than one-half the biological uncertainty, then it is contributing insignificantly (less than 20 percent) to the total uncertainty of laboratory results. It should be emphasized that the Aspen goals consider only intralaboratory components of precision: long term for group screening and short term for individual testing. Neither interlaboratory nor intermethod components of precision are specified. In individual single point testing, the discrimination levels or reference points which separate disease from nondisease are not defined. Furthermore, interlaboratory comparability will depend on the development of accuracy specifications for these reference points. Finally, if the precision goal for an analyte were to become more stringent than is required for clinical application, it is recommended that medical judgement take precedence in setting the analytical goal.

C. In the improvement of today's clinical laboratory technology and in the development of new technologies, special consideration should be given to:

1. Adequate definition of the chemical substance to be measured (analyte).

2. Preanalytic factors such as specimen collection and transportation.

3. The intraindividual and interindividual biologic variability.

4. Turnaround time and STAT capability.

5. Specification of the clinical application.

6. Economic cost/benefit ratio.

7. Patient risk/benefit ratio.

8. Deviation from the true value.

Comment

An analytic result is the end product of a number of systematic steps which include preanalytic as well as analytic factors. In certain instances, the uncertainty introduced by either factor may dominate the analytical system. Adequate definition of the analyte will lead to selection of appropriate methodology which will avoid potential matrix and other interferences. Preanalytic variability should be either minimized or clearly understood in order to interpret test results correctly. This will require education of clinicians, patients, and dietary and phlebotomy personnel. To increase our knowledge of biological variability will require funding; such studies should be carried out and published promptly. Turnaround time and STAT capability impacts instrument design, reagent availability and reliability, and personnel qualifications. Specification of medical usefulness requires thorough knowledge of the clinical situations in which an analytical result is used by a physician. Economic cost/benefit ratio requires

implementation of cost analysis techniques and a realization of the economic impact of both false positive and negative results. By distinguishing another factor, patient risk/benefit is emphasized as a separate area of study. Re-emphasis of the need to quantitatively define the interval between the analytical result and the true value ties in with the accuracy specifications of Recommendation A.

D. Intermethod bias should be quantified.

Comment

The basis of intermethod comparison is analysis of a known analyte in a known matrix by each method according to a well designed protocol. Each method will result in values with unique statistical parameters. The difference between these parameters, i.e., the respective mean and confidence intervals, should be quantified as statements of both transferability and accuracy. Statements of intermethod bias should be related to the true value.

E. Reference materials should be used to provide calibration and verification of laboratory measurements.

Comment

While laboratory measurements may be considered to be both calibrated and verified by reference materials because they provide a basis for measuring random analytic imprecision and constant systematic bias, considerable research and development is necessary before such materials can be used to study random systematic bias. Presently, the only acceptable way of measuring the bias* of one method relative to another is by simultaneous measurements of a large number of different patient specimens over the range of the method.

*The average bias caused by miscellaneous unknown interferences plus inherent methodological bias.

F. There is a major need for development of methods for:

1. Determination of reference values in health and disease.

2. Determination of disease prevalence.

3. Setting priorities for analytical improvement.

Comment

Reference values for an analyte in healthy and diseased individuals are more comprehensive and useful than simplistic statements of "normal ranges". Reference values permit more meaningful statements of the clinical usefulness of a method, particularly in individual single point testing where the patient's value is compared with a reference point. In this context, probability statements can be made for the clinical sensitivity and specificity of the result. Knowledge of the prevalence of a disease in the population is necessary to set the discrimination point in the context of group screening, so that economic costs and/or patient risk functions are minimized. Because limited resources are available for the implementation of all of these recommendations, it is necessary to establish priorities by analyte. However, the total picture must be evaluated, i.e., technical feasibility, cost, education requirements, etc., in making priority decisions.

G. There is a major need to improve laboratory reporting techniques and to promote the understanding of the clinical use of laboratory data.

Comment

There is little knowledge of how frequently a clinician recognizes unexpected abnormal laboratory results and the subsequent action which is taken. Such information is necessary in order to understand the best reporting formats for laboratory information, algorithms for the selection of appropriate tests in the medical setting, and programs for concurrent interaction between clinicians and clinical laboratories. This area impacts on laboratory cost and patient risk. There is preliminary evidence that recognition of these

abnormalities may have a beneficial medical impact, although the economic cost could increase because of the number of followup tests.

H. There is a need to adopt a universal vocabulary of analytical terms.

Comment

The ability to communicate in a common language will improve both medical and technical understanding of disease processes and the transferability of laboratory data and information.

GROUP 1.2 Organizational Component

Chairman: Dr. Joseph H. Boutwell (CDC)

Participants:
Dr. Gerald R. Cooper (CDC)
Dr. Dale Fisher (FDA)
Mr. J. Paul Cali (NBS)
Dr. Pierre Keitges (NCCLS)
Dr. Nathan Gochman (AACC)
Dr. Rex B. Conn, Jr. (ASCP)
Dr. George F. Grannis (CAP)
Dr. Roger K. Gilbert (CAP)
Mr. William T. Ryan (Industry)

Observers:
Dr. Stanley Brown (International Federation of Clinical Chemistry)
Dr. Ronald Elin (NCCLS)(NIH)
Mr. Rodney Munsey (Pharm. Mfg. Assoc.)
Dr. John O'Rangers (FDA)
Dr. Aubrey Outschoorn (USP)
Mr. Rodger Richeal (SYVA)
Mr. Jaxon White (Health Industries Mfg. Assoc.)
Professor Dankwart Stamm (German Assoc. of Clinical Chemistry)
Dr. John Ponzo (Ames Co.)
Dr. Wolfgang Gruber (Germany)
Dr. Jonathan Miller (Abbott Co.)
Dr. Edward R. Kimmelman (DuPont Co.)
Dr. Richard G. Nadeau (NCCLS)
Mr. F. Kirk Nelson (Am. Hosp. Supply)
Dr. George F. Grannis (CAP)
Ms. Helen Hargadon (Devices & Diagnostics Letter)
Dr. William Knouse (CDC)

Assignment

To design an organizational plan that will effectively and efficiently use the available national resources of both government and private sectors. One aspect will be improvement of communications between the professions and promotion of group scientific investigation.

Initial Agenda

1. Position Paper Summary Statement by each of the Participants

2. Definitions and Relationships

   Analytical goals - analytical accuracy, specificity, sensitivity, and precision.

   Clinical goals - clinical specificity and sensitivity.

   Analytical methods - field (practical or routine) reference, and definitive.

   Reference materials - calibrators and controls.

3. Participant Discussion Topics

   Basic Research
   Analytical Method Development
   First Stage Consensus Development
   Validation Testing
   Transferability Testing
   Second Stage Consensus Adoption
   National Consensus Appraisal and Approval
   Transfer Mechanisms
   Priority Setting (Medical Need Basis)
   Periodic Review and Updating

REPORT OF WORKING GROUP 1.2

Group 1.2 commenced its deliberations with a brief statement by the chairman who emphasized the problems of topic overlap with other working groups, and the interactions to be expected. He briefly outlined, as shown in the agenda, the functional components in the development of a reference method and his proposal that the group discussion should lead to decisions as to how these functions could or should be most appropriately assigned or accepted.

The chairman called for initial statements from each of the participants, each of which was immediately followed by brief discussion. The main points emphasized by each

participant will be noted together with a brief summary of the following discussion.

Dr. William T. Ryan - Industry

Dr. Ryan agreed that there was a need for a well-understood national system by which reference methods were developed. He felt that this Conference had brought together the appropriate representatives of those concerned. He suggested that the many diverse interests represented should be concerned with how such an undertaking should be carried out. He raised a question concerning any agreements "to do" or "not to do" as to whether they might be interpreted as "combination in restraint of trade."

Discussion: Dr. Cooper preferred the duties and responsibilities to be "assumed", rather than "assigned and accepted."

Dr. Keitges preferred that any "framework" for such a system be developed in the private sector, rather than by government. Dr. Ryan agreed. There was general discussion concerning the need to preserve individual and group initiative and creativity in any system which was agreed upon.

Dr. Roger K. Gilbert - (CAP)

Dr. Gilbert recognized the Conference as a unique opportunity to discuss these related problems, and to perhaps reconcile two divergent streams of concern about the effect of formalizing reference method development. He pointed to one group who believe that inaccuracy is a major contribution to dispersion of clinical laboratory results and therefore that the reference method and materials development system must be improved and another group which believes that a formal structure will become a greater burden than its benefits can justify and will serve to inhibit advancement in the field of clinical chemistry. Dr. Gilbert expressed the view that a factual case for a serious inaccuracy problem had not been made and that national priorities and programs should be based on factual data.

Dr. Gilbert further expressed his belief that laboratory resources would be more properly and effectively directed to the solution of other problems. He further raised the

question as to the use to be made of these reference methods and pointed to the development and proposed application of the hexokinase reference method for glucose and its publication in the Federal Register in connection with a proposed product class standard for glucose in serum. He emphasized the dangers of regulation.

Discussion: Mr. Cali (NBS) disagreed with Dr. Gilbert on the importance of accuracy in clinical laboratories and emphasized the importance of the establishment of a national measurement system in clinical chemistry based on accuracy, and on reference methods and associated reference materials.

Dr. Fisher (FDA) commented that the problem of definition of "accuracy" may explain differences in viewpoint. The point is the need to control error, both accuracy (bias) and precision. Over a long term, accuracy and precision blend together in that fluctuations in bias are part of long term precision. He further emphasized that to track effects on results due to inevitable differences and changes in methods, independent accuracy statements must be made.

Dr. Rex B. Conn - (ASCP)

Dr. Conn emphasized that the primary objective must be (ultimately) improvement in patient care, but the issue of costs cannot be avoided. He recommended that our group attempt to define a process and not a state; that if all of us had common definitions, sufficient data, and the basis for appropriate cost/benefit analysis, we might not need a consensus process for answers. He went on to indicate that an Information Exchange was needed in the reference method and materials area because space is too limited in professional journals, and to encourage the application to specific problems by individual initiative and creativity.

Discussion: Dr. Cooper indicated the need to individualize problems; that needs for reference methods and materials differ with the medical problem, the indicator analyte, and the state of medical and analytical knowledge.

Dr. Nathan Gochman - (AACC)

Dr. Gochman indicated AACC support for the present "non-system" but that it needed centralized coordination because of large costs and resource limitations of those

involved. He emphasized the need to base clinical laboratory measurements on "true values," to insure measurement compatibility (although some "arbitrary" decisions or definitions will be needed.) He asserted that the proposed reference system should include the concept of "traceability" to definitive methods (those of undisputed accuracy).

Dr. Gochman also proposed that the "system" include the acceptance of "SI units," and uniform nomenclature, and that such uniformity should extend internationally; and the developed system should in its control and organizational structure, reinforce the voluntary standard consensus system, as opposed to a quasi-governmental system. The NCCLS was proposed as providing an appropriate central decision arena. He stated that professional societies would necessarily provide major inputs to the system, especially as to specific needs and proposed methods and materials. He further emphasized that the voluntary consensus system depends on vigorous debate and spirited dissent. He referred to the AACC position paper proposing specific functions for government agencies.

Discussion: Dr. Keitges raised a question as to whether these government functions were indicative or exlusive. Dr. Gochman stated that he felt these were indicative of competencies and past history. Mr. Cali pointed to the long history of NBS in its policy of working with industry. Dr. Fisher indicated that FDA does not regard the proposed system as a direct part of the regulatory process. Dr. Gilbert raised the question as to how priorities and actual needs were to be established and related to costs and priorities. He was concerned that unless this were carefully addressed, an unbalanced emphasis of the reference system on the quality of laboratory services might be the result. General discussion with examples of specific needs ensued.

Dr. Pierre Keitges - (NCCLS)

Dr. Keitges recognized the need for consensus standards in the area of reference methods and materials as well as the need for definitive methods. In view of the limited resources available, it is indeed important to define the criteria for priority setting and he felt that there was no disagreement that medical usefulness and improved patient care must be included, along with cechnical capability.

Dr. Keitges emphasized the capability of NCCLS to serve national needs as a voluntary system, able to come to consensus on priorities and approaches, and to convene those who can assign resources to solve the problems as they are clearly seen. He emphasized the need for coordination, communication and the need for an information repository.

In addition, Dr. Keitges felt that we should consider standards of practice, and testing of transferability and in the long run, there should be an on-going evaluation of the impact of reference methods and materials; i.e., the utility of our efforts.

Discussion: Dr. Gochman commented that excessive emphasis on the undefined concept of medical usefulness can deter us from the problem of lack of standards. Medical usefulness must be a dynamic term, and not used to pre-judge the information level needed.

Dr. Gilbert supported the concept of NCCLS as a decision arena. CAP standards have been and will in the future be submitted to NCCLS for adoption.

Dr. Cooper questioned how NCCLS would use medical usefulness to set priorities.

Dr. Keitges referred to the work of the Area Committee on Toxicology whose product to date is priorities; and only now area subcommittees are to be appointed.

Dr. Cooper indicated a concern with NCCLS serving as both developer, advocate, and judge to determine the acceptability of reference methods and materials.

Dr. Keitges agreed that the NCCLS mechanism must be carefully used to assure this separation; and that a clear separation must be made between development of proposed consensus standards, and assessment of consensus.

Mr. Paul Cali - (NBS)

Mr. Cali described the NBS approach to measurement compatibility and emphasized that it has been shown to work. Not all health measurements can be subjected, at least at present, to an accuracy statement. Some will be related to

an artificial or arbitrary base. He emphasized that NBS is not regulatory but does work with regulatory agencies to develop the measurement base, which they may require. NBS works where they are welcome, such as ASTM and NCCLS, and where cooperation is offered. The payoff has been great.

Discussion: Dr. Gilbert emphasized that the required levels of accuracy may differ. He also referred to a request for assistance CAP made to NBS some time ago in relation to the "accuracy" of temperature measurements.

Dr. Keitges inquired as to implementation or application of the measurement process after it has been developed. Mr. Cali responded that NBS has no direct responsibility but works closely with ASTM.

Dr. Cooper asked, "What is really bothering everyone?", and suggested that it was possibly the end use of the reference method.

A general discussion on this topic ensued. It included the suggestion that, for example, there might be an unwise reimbursement requirement for particular methods. Mr. Cali suggested that the principle of "equivalency" of method results adopted by the EPA would be advisable. Any requirement would be in relation to intended use and that reference methods and materials are needed for assessment and improvement of field methods and materials. FDA is concerned with error control and traceability on a long term basis.

Dr. Gochman commented that anything invented can be abused but this should not inhibit the invention of reference methods.

Dr. Gilbert asked, will the proposed system have priorities or will all the squares be required to be filled?

Dr. Dale J. Fisher - (FDA)

Dr. Fisher stated the FDA does not intend to design or use the NRS as a regulatory system. FDA does need a scientifically developed accurate measurement base, and reference methods and materials as well as information about medical needs for performance in order to assess products. There is a need to control errors associated with such

assessment and determine the contribution of product error, both precision (standard deviation) and accuracy problems (bias relative to a standard). Accuracy is needed both over space; from method to method; lab to lab; and from time to time; all of which require a stable defined common base. He suggested that the Conference take positions about participation by other concerned groups; a newsletter; another meeting and any future activities.

A measurement base for clinical chemistry has begun to be developed through the joint efforts of the organizations represented here and must be expanded and strengthened. We are here to identify ways to do this. We need reference materials and methods that are acceptable to and used by clinical researchers, manufacturers, and clinical laboratories. FDA intends to work with all interested parties to improve clinical measurements. FDA will welcome and seriously consider such inputs from the NRS.

Discussion: Dr. Ryan had a question about FDA's position on the reference system. Industry generally felt that FDA would use the reference system in a regulatory way. Dr. Fisher referred Dr. Ryan to the first two pages of the completed FDA draft position paper (which Dr. Ryan had received at the Conference, but not prior to the meeting) which indicates that FDA will consider inputs from all such sources, and use them as deemed appropriate.

Dr. Gilbert questioned the need for reference methods in the FDA product appraisal system.

Considerable group discussion followed, revolving around the FDA "safety and efficacy requirements" and their exact meaning when applied to diagnostic laboratory products. In response to a question, Dr. Fisher indicated that if all field methods give the same answers for specimens, FDA would have no problem if the answers are accurate and reliable enough for medical needs. Dr. Gilbert raised the question "What if all methods are inaccurate but give the same answer?" Dr. Fisher replied that the FDA position and action would have to be based on medical needs; i.e., if the inaccuracy were medically misleading and related to a specific product or group of products, FDA would probably consider an action, depending on the degree that accuracy and

precision would be required to different degrees in different products.

Dr. Conn was concerned that FDA actions and reference method adoption (even if by a consensus procedure) would lead to the exclusion of (useful) methods even if they were equivalent in performance to existing standards. Dr. Fisher replied that FDA is being especially careful to write its requirements in terms of identifying and stating equivalent performance characteristics and specifications that are needed to assure safe and effective use.

Dr. Gerald Cooper - (CDC)

Dr. Cooper indicated that the Clinical Chemistry Division had recognized reference methods and materials as essential components for laboratory improvement in clinical chemistry as it had been for other laboratory disciplines. CDC interest in helping to initiate and sponsor this Conference lay in the recognition by CDC that a reference system must be adopted by consensus, and the processes involved must be understood by all involved. Dr. Cooper felt that the development of guidelines for the interactions in such a system required an understanding by the organizational components here represented of the process itself and the responsibilities assumed by a contributor to the national system. He supported Dr. Gilbert's emphasis that it is necessary to "count the cost!" He went on to emphasize the need in a reference system for three points: viz., the scientific base, consensus acceptance and a way to transfer the accuracy base once a reference system was adopted.

Discussion: Dr. Keitges: Do you see a need for a new system or does the present (non) system simply need modification?

Dr. Cooper explained that he felt that the present unstructured system, only partially understood should not be discarded. A totally new super-structure is not needed, but a great deal more understanding is needed, as well as some modifications to minimize excessive multiple duplicative activities and to provide a clear path to national consensus acceptance of reference methods and materials.

Dr. Ryan wondered what does CDC really mean? Dr. Cooper's

statements seem to clash with the CDC draft position paper. Dr. Cooper's reply is that the draft position represents CDC's interests, its estimate of needs, and its willingness to assign resources.

Dr. Gilbert expressed his feeling of uneasiness with the CDC (governmental) program of reference method and material development. He went on the criticize again the lack of specific documentation of specific needs. Dr. Cooper agreed that the CDC draft document did not contain data and that he did not feel that it was appropriate to discuss needs in detail. He felt that previous discussion had led to an agreement (certainly he agreed) that limited resources indicated that priorities had to be developed on the basis of demonstrated needs. The CDC program had been developed in this way.

Dr. Cooper emphasized that one of the compelling reasons for CDC initiative and sponsorship of this present Conference was the recognition of the need for priority setting on the basis of national need and the need for a well recognized and well understood National Consensus System for reference methods and materials.

Dr. Keitges indicated that he also read the CDC draft position paper as seeking the central management of the reference system. Dr. Cooper responded again that it was intended to show CDC concern for accuracy, for a consensus system and its willingness and capability.

Observer Comments:

Dr. Stanley Brown (England) - (International Federation for Clinical Chemistry)

Dr. Brown emphasized the need for any U.S.A. national system to include a consideration of impact internationally, and early interaction with appropriate international and other national bodies can prevent later problems. He felt that outsiders can offer a critical insight important to the national system, such as international transferability testing.

Dr. Dankwart Stamm (W. Germany) - (German Association of Clinical Chemistry)

Reliability is important and even more so is comparability. To the extent it is achieved, it will be cost beneficial in patient care, in teaching and in research. The best basis of comparability, and the least expensive, is accuracy. It must be used not only for standardized field methods but for country-wide quality control for accuracy.

Dr. John O'Rangers - (FDA)

It is important to consider the accuracy problem, especially with new emerging techniques. In legal aspects of regulation, any person can express his views (or his group's views) within the legal mechanism. Eventually the government will become involved in a regulatory way or through medicare payments, and it is important for the private sector to express itself in terms of medical usefulness, and research, and cost effectiveness. He did not feel a newsletter would be effective and recommended that we consider a second meeting with interim work done on national goals.

Mr. Jaxon White, Jr. - (HIMA)

Mr. White indicated that he and others had explored the question of "combination in restraint of trade" and did not feel that it offers any problem in this area.

Dr. Ronald Elin - (NIH)

Dr. Elin indicated that he was confused by an apparent dichotomy which stressed the benefit to the patient but spoke to accuracy as a major thrust. We must define the real needs first and accuracy is not necessarily a compelling need. While it seems to be a goal that a plan is to be designed, he has heard no such plan proposed.

Dr. George Grannis - (Ohio State University)

Dr. Grannis posed three questions. To Mr. Cali: How can "equivalency" be determined? To Dr. Fisher: What is the need FDA has for reference methods? Can these needs be fulfilled in other ways?

Dr. Jonathan Miller - (Abbott)

There is a need to consider the definition of minimum clinical needs. There are always trade-offs in developing methods between specifications and costs; between benefits

and costs; and cost of the system will affect the patient.

Dr. Aubrey S. Outshoorn - (U.S. Pharmacopoeia)

Dr. Outshoorn drew to our attention the fact that the United States Pharmacopoeia has a quasi-legal status and that its standards of drug identity and purity, etc. are legally enforceable. Reference standards are available.

Observer Written Comments

Dr. Stanley Brown (England) - (IFCC)

1) IFCC is only one of several international bodies with interests in Reference Methods and Materials (R.M.M).

2) IFCC has been concerned since 1973 to coordinate activities in the field, and apply tested principles worldwide--especially to improve performance in second and third world countries.

3) U.S. should not underestimate its scientific impact on the world scene--but there should be two-way interaction.

4) U.S. R.M.M.s will benefit from international tests of transferability--outside bodies free and able to challenge the (scientific) positions of prestigious U.S. centers.

5) R.M.M.s are of fundamental importance in validating the performance of multi-channel analysers; perhaps fewer, better and more meaningful measurements are desirable?

6) R.M.M.s achieved so far, should be taken as models for "difficult" analytes, such as trace metals, steroid, thyroid, peptide hormones--not amenable to consensus approval.

7) U.S. is necessarily concerned with organizational/ regulatory aspects of R.M.M.s, but scientific and medical aspects predominate on the international front.

8) Principles of R.M.M.s in clinical chemistry should be extended into other areas of clinical laboratory science.

Dr. Aubrey S. Outschoorn - (USP)

If a reference method, and with it as necessary the appropriate reference standard, has been properly validated on the basis of technical acceptability for a particular test, USP has a mechanism for rapid publication and dissemination. It can, for instance, be published in Pharmacopoeia Forum and if no objections are received, included in the next Supplement to USP (published annually). Identification in the compendia or a Supplement would make the method obligatory for the particular item, e.g. a laboratory test kit. It would be required, however, that the USP Committee of Revision make a prior evaluation of the recommendations of the method and adoption of the material as a USP Reference Standard, before such publication.

Dr. Joseph Boutwell - (CDC) - Chairman

Dr. Boutwell responded to previous questions as to the purpose of the Conference and why was it taken for granted that accuracy was an important problem which should be solved by a national reference system.

First, a large effort is now being devoted both in the public and private sectors to the development of reference methods and materials. The CDC motivation for the Conference has been well expressed by Dr. Cooper as concern for the accuracy problem, concern for the use of a national consensus system to achieve acceptance of reference methods and materials and concern for effective use of resources on high priority projects.

Dr. Keitges lead off a general discussion of the problem of defining needs for accuracy. Is CDC spending too much on reference methods? There was general agreement on the need to collect and analyze the data which already exists.

The general discussion at this point led to several problems of definition and understanding: What is the operational meaning of "consensus?" (Dr. Ryan) Dr. Keitges (as President-elect of NCCLS) suggested that a working definition was really the end product of a process (the NCCLS process?) which results in a written specification "which all can live with." Mr. Cali added that the integrity of the process must be able to stand up to a legal challenge.

Dr. Elin pointed to the working group agenda and emphasized the importance of the different meanings of "analytical specificity and sensitivity" and "clinical specificity and sensitivity."

Dr. Boutwell reviewed the functional components in the agenda. Two additional steps were added. (1) Priority setting on the basis of medical need; (2) Periodic Review and Updating.

The Working Group then discussed the way in which recommendations should be developed. After some discussion as to alternatives, the chairman proposed that a list of topic areas be developed. Further discussion ensued on the need for priorities, regulatory impact, and manufacturer reference base. After this, the following list of topic areas was developed by the group.

1. Consensus process
2. Priority listing
3. Basic Research
4. Communication
5. Overall concept
6. Validation testing
7. Transferability testing
8. Identification of Resources
9. Assumption of Responsibilities
10. Revision and Updating process
11. Coordination
12. Nomenclature
13. Assessment of Effectiveness

These topics were chosen to orient and categorize Proposed Recommendations. The participants working in several different groups assumed the responsibility to submit formulated statements of Recommendation which were submitted and debated on Thursday, November 17, 1977. The Recommendations were to deal with any or all of the following aspects--organization, individuals, freedom, interaction and process.

Thursday, November 17, 1977

Each of the twelve submitted recommendations was read. After a short break, during which time the first three were copied for the participants, each recommendation in turn was debated and voted. Each approved recommendation was approved without a dissenting vote; for those recommendations which were not adopted, that action was taken by unanimous consent.

The specific action taken on each recommendation follow the text.

RECOMMENDATION 1.

BASIC RESEARCH

The very nature and definition of basic research implies freedom to select the problem and to use whatever approach the scientist(s) thinks best. However, within the proposed organizational structure there should exist a mechanism for the identification of basic research measurement problems (arranged, if possible, in priority order) that could serve the basic research community as a guide for the selection of research projects. This aspect should be incorporated within the communication network segment of the organization.

Action taken: Approved as submitted.

RECOMMENDATION 2.

RESOURCES

There are two aspects to the question of resources: (1) Identification of current resources devoted to contribution to central coordination for an NRS; and (2) A mechanism to recruit additional resources.

(1) Identification of Current Resources

There are national resources currently committed to support of an NRS. Some of these are legislatively mandated; (e.g., NBS has legislative responsibility to provide SRM's, and United States Pharmacopoeia standards have a legal standing). Others are mandated as falling within the perceived scope and responsibilities of various organizations (e.g., NCCLS exists to provide a voluntary consensus mechanism for clinical standards). A mechanism should be put in place to identify these resources, to obtain an estimate of current and (likely) future magnitude in terms of manpower, facilities, and dollars. In other words, a national inventory should be made and a mechanism put in place to review and update periodically.

(2) Mechanism to Recruit Additional Resources

It can be taken as a given that current resources will be insufficient to undertake all identified and high-priority items. Some part of the organization should be given the task of addressing this problem. Possible sources of resource enhancement are:

(a) Government support directly or indirectly through appropriate agencies.

(b) Professional societies.

(c) Trade associations or individual manufacturers.

(d) Self-support by sale of documents.

Action taken: This recommendation was approved after minor additions to the original draft.

RECOMMENDATION 3.

OVERALL CONCEPT

The Conference endorses the concept of improved coordination of the components of the national reference system in clinical chemistry, so that they can be more effectively applied. The capabilities are currently provided by a number of organizations, including governmental agencies, voluntary societies and industry. The Conference believes that the involvement and commitment of these diverse groups is a principal source of strength.

In order to enhance effective and economic application, coordination should be strengthened. The coordination should be through a voluntary consensus organization in order to ensure open decision-making, effective communication, identification of needs and development of priorities.

The NCCLS was established to accomplish these objectives, and the Conference looks forward to NCCLS effectively fulfilling this role.

The Conference believes that the sponsors and cosponsors should reaffirm their commitment to the consensus mechanism through NCCLS.

The Conference recommends that NCCLS develop a plan to implement these recommendations and submit the plan to its constituent organizations and other appropriate parties.

Action taken: Approved as submitted after minor modification.

(DRAFT) RECOMMENDATION 4.

NOMENCLATURE

The basis for nomenclature for the National Reference System in Clinical Chemistry should be the Glossary provided in the AACC Position Paper. This Glossary was compiled from several documents in current national and international use. Further revisions are to be expected as a result of review by other bodies.

Action taken: Not adopted.

(REVISED)
(SUBSTITUTE) RECOMMENDATION 4.

NOMENCLATURE

The Conference considers that there is a need for improved clarification of terminology. The NRS process is directed to develop a Glossary compiled from the several documents in current national and international use, as well as from the position papers presented at this Conference. This Glossary is intended as operational terminology for the NRS process, and will be revised as a result of continual review.

Action taken: Adopted as revised (from draft recommendation 11).

RECOMMENDATION 5.

CONSENSUS AND COMMUNICATION

It is recommended that the NRS be implemented through a coordinating body that embraces the following aspects of consensus and communication:

1. It will solicit or accept candidate projects based on documented need for the objective. These projects will be subjected to an organized consensus process to establish priorities.

2. The consensus process will develop priority listings and it will be communicated through publication to the clinical laboratory community.

3. It will receive any candidate reference method and/or material from the laboratory community and the consensus process will evaluate such methods and/or materials proposals for scientific integrity and practicality in terms of resources. With consensus approval at this point, the system progresses to validation testing.

4. The validation testing results will be published in a suitable publication(s).

5. The sponsoring organization or individual(s) will respond to the comments that are solicited by the publication. The coordinating body of the NRS will determine if all comments have been addressed and if the candidate reference method and/or material may proceed to transferability testing.

6. The results of transferability testing will be published.

7. The sponsoring organization or individual(s) will respond to all negative comments that may result from the publication exercise. A coordinating body of the NRS will then determine if all such comments have been properly addressed and then it will submit the candidate method and/or material to the complete NRS structure for consensus approval to be completed within a defined

period of time.

8. It will maintain a repository of all publications, exchanges, and documentation that are associated with the procedures of the NRS.

9. It will be responsible for continued evaluation of the utilization and assessment of effectiveness of the approved methods and/or materials and, if indicated, will conduct revision, updating, or revocation.

10. Based on circumstances, a candidate reference method and/or material may be introduced at appropriate points in the system or suspended from further consideration within the system with the approval of a coordinating body of the NRS.

Action taken: Adopted after minor modifications.

RECOMMENDATION 6.

IMPLEMENTATION OF CONSENSUS AND COMMUNICATION

Having reviewed the various recommendations of Working Group 1.2, it is concluded that the present structure of the NCCLS, with necessary augmentation, organization modification, and development of resources, can fulfill the objectives and goals of the NRS pertaining to consensus, coordination and communication.

Action taken: Adopted as revised.

RECOMMENDATION 7.

VALIDATION TESTING

When it is proposed that validation testing be assumed for a reference method or material, the following should be done:

1. There will be a consensus review under NRS to assure that priority of need and technical feasibility coexist, and that an adequate description of the proposed

reference method or material has been proposed.

Scientific evidence or widespread consensus about medical need and priorities must exist before validation testing is implemented.

Alternatives for review are: (1) A broadly based consensus review organization such as NCCLS; (2) a study group of a professional society, or co-sponsoring societies; or (3) the institution itself that expends the resources, with or without an ad hoc committee.

When it has been decided to undertake validation testing, a notice should be submitted immediately to the NRS communication system.

2. A validation protocol appropriate to the particular analyte method or material shall be submitted for a consensus judgment that at least the minimum requirements for validation will be met.

   Alternatives for review are: (1) A broadly based consensus review organization such as NCCLS; (2) a study group of a professional society or societies; or (3) the institution itself that expends the resources with an ad hoc committee.

3. Validation testing. The institution carrying out the test is solely responsible.

4. An appraisal of the validation will be carried out by a broadly based professionally competent consensus group for the particular item to see if it is scientifically sound.

   Alternatives for review are: (1) A broadly based consensus review organization such as NCCLS; (2) a study group of a professional society or societies; or (3) an ad hoc scientifically competent study group of the institution itself that expend the funds.

Action taken: Adopted.

RECOMMENDATION 8.

TRANSFERABILITY TESTING

1. Assuming that validation has been judged to be adequate, the governmental institutions and professional groups that will assume responsibilty to carry out transferability testing will develop a protocol and criteria for successful transferability.

2. For the formal NRS process, the prepared transferability protocol for the particular analyte method or material will be submitted for a consensus judgment that at least the minimum requirements for transferability will be met.

   Alternatives for review are: (1) A broadly based consensus review organization such as NCCLS (2) a study group of a professional society or societies; or (3) the institution itself that expends the resources with an ad hoc committee.

3. Transferability testing. The institution involved in carrying out the test is solely responsible.

4. An appraisal of the transferability testing will be carried out by a broadly based professionally competent consensus group to see if it is scientifically sound.

   Alternatives for review are: (1) A scientifically competent group of NCCLS; (2) a study group of a professional society or societies; or (3) an ad hoc scientifically competent study group of the institution itself that expends the funds, professional societies, and government groups.

5. After transferability testing has been proved successful, the institution carrying out the studies will submit a proposed reference method or material to NCCLS for their consideration of consensus approval.

6. When it has been decided to submit the proposed reference method or material to NCCLS for their consideration for consensus development, a notice will

be submitted immediately to the NRS communication system.

Action taken: Adopted.

(DRAFT) RECOMMENDATION 9.

COMMUNICATION

a. Review the addition of organizations as observers or participants.

b. Establish a newsletter consisting of reproductions of written materials as submitted by concerned parties. Encourage feedback of comments for later issues.

c. Provide a mechanism for another meeting after the proceedings of this meeting have been digested, and after all groups have had time to interact with the other groups of the Conference.

d. Recommendation: Before this meeting adjourns, it is agreed that another meeting will be held.

e. Site of NRS Communications System. A possible site is CDC.

Action taken: Not adopted.

RECOMMENDATION 10.

ASSUMPTION OF RESPONSIBILITY

It was recognized by the Conference that individuals, professional scientific associations, industry, government agencies, consensus organizations, and international and regional organizations may assume any of the necessary functions leading to the development of a reference method or material.

Action taken: Adopted as revised.

RECOMMENDATION 11.
See RECOMMENDATION 4.

(DRAFT) RECOMMENDATION 12.

STATISTICAL MODEL OF ERROR

The Conference considers that there is an urgent need for the development of a statistical model of error for quantitative analyses in the clinical chemistry laboratory. The model should consider the inter-relationships of the various sources of error and their impact on the analytic output of the laboratory.

When the model has been developed, an attempt should be made to estimate the size of the individual components of error for a selection of analytes. Data should be drawn from all available sources.

An attempt should be made to learn the effect on the error of the analytic output of changing the size of the individual components of error in order to identify the limiting factors.

Action taken: Not adopted.

(REVISED) RECOMMENDATION 12.

SOURCES OF ERROR

The Conference recognizes a necessity for the development of priorities for reference methods and materials to identify and characterize the sources of error through a statistical study of existing performance evaluation data.

Action taken: Revised and adopted.

TOPIC 2. Materials Component of the National Reference System

Topic Coordinator: Dr. Thorne J. Butler (ASCP)

a. Orientation, Procedural Instructions and Guidelines

b. Joint Session on Nomenclature

(1) Discussion of basis of a system (purity, intended application, degree of characterization, etc., or a combination?)

(2) Applicability of proposed terms (position paper definitions, and others)

The meeting was opened in a joint session with the Biological Materials Working Group. Dr. Thorne Butler's remarks are summarized as follows:

Although definitions are ultimately important, we should not spend undue time refining definitions at this Conference. What is very important is that we establish technical criteria for preparation and use of reference materials. Dr. Butler raised the issue of whether the class of Certified Reference Materials includes or excludes the biological matrix reference materials and should the latter also be designated for use in a National Reference System for Clinical Chemistry (NRSCC). He suggested that three types of reference materials be designated. These should be denoted as Certified Reference Materials. He suggested that the working groups define these terms according to their degree of characterization and their intended use.

GROUP 2.1 Standard Materials

Chairman: Dr. George Uriano (NBS)

Participants: Dr. Richard J. Carter (CDC)
Dr. Nabeeh Mourad (FDA)
Dr. Theodore Peters, Jr. (NCCLS)
Dr. Royden N. Rand (AACC)

Dr. John A. Lott (ASCP)
Dr. Daniel J. Hanson (CAP)
Dr. Thomas Adams (Industry)

Observers:
Mr. Louis Alexander (CDC)
Dr. Irvin Asher (FDA)
Mr. Roger Barnes (FDA)
Dr. Richard Bonder (Worthington)
Mr. John Daly (Technicon)
Dr. William S. Knight (Beckman Instruments)
Ms. Hortense Macon (FDA)
Mr. Thomas Mears (ACS)
Dr. D. L. Petitijean (Perkin-Elmer)
Dr. Paul Schneider (Bio-Dynamics)
Dr. Morris Suggs (CDC)
Ms. Frances Wherry (FDA)
Mr. Timothy White (Nobel Scientific Industry)
Mr. Arthur Zarchy (Mallinckrodt)

Assignment

To outline and partially specify the criteria for selection, validation, characterizing, and labeling of such materials and additionally consider priority needs for developing specific standards.

Initial Agenda

1. Purposes and Uses of Standard Materials

   - Method Development and Evaluation
   - Production (and evaluation) of other materials
   - Calibration, and Controls

2. Specification Criteria

   - Technical specifications. Accuracy, purity, (primary analyte, isomeric forms, matrix and trace impurities), form (pure vs. matrix), coherence (with natural specimens), stability, homogeneity, interferences, special characteristics.

   - Application of Specifications.
     By classes or by analyte?
   - How are specifications to be developed?

3. Validation and Certification

   - Testing protocol and performance limits
   - Administrative procedures
   - Consensus review
   - Independent validation?

4. Labeling and Use(s)

   - Labeling requirements
   - Priority of needs for new lots and new materials.
   - Certifying and Validating new Lots
   - Usage Restrictions?

5. Priority Setting for Materials Development

REPORT OF WORKING GROUP 2.1

Summary Notes

The Standard Materials Working Group session began with each delegate making a brief statement of the general views of the organization, which he represented.

Dr. Adams (Industry) started out by commending the National Bureau of Standards (NBS) for its past work in the development of Standard Reference Materials (SRMs) and requested that such efforts be accelerated. He emphasized that reference materials should be produced in a form suitable for intended use. Industry will continue to produce reference materials. Government-produced reference materials should not unfairly compete with those produced commercially. He emphasized that a prime criterion for development of reference materials should be Good Manufacturing Practice (GMP) and that reference material production should always be related to medical needs.

Dr. Mourad (FDA) stated that one of FDA's responsibilities is to regulate industrial producers of in-vitro diagnostic testing products. Reference materials are necessary because they are an integral part of laboratory performance. Reference materials must be produced on a timely basis, taking into account priorities already established through FDA mechanisms (e.g., advisory panels) and be based on

medical need. The producer of the reference materials is not the critical factor but rather the quality of the reference materials is the most important consideration. The question of definitions and also reference materials validation procedures should be addressed by the panel.

Dr. Peters (NCCLS) referred to recommendations contained in the NCCLS position paper to describe the role of NCCLS in the voluntary standards process.

Summary of NCCLS Recommendations:

> Standards of practice and of methods and materials in clinical chemistry must be based on medical usefulness and clinical relevance. The development, review, and updating of such standards must be carried out through the process of peer review in a consensus environment.
>
> Each organization and individual within the NRS must be encouraged to contribute to the standards development process based on ability and resources.
>
> NCCLS should serve as the communication mechanism in the development of goals and priorities for the standard making process.
>
> NCCLS should serve as the forum for member organizations of the NRS to coordinate the development of standards.
>
> NCCLS should provide the initial and ongoing consensus review of NRS generated standards.
>
> NCCLS should act as the repository of NRS standards.

Dr. Peters stated that NCCLS sees its role as providing a mechanism for the development and implementation of consensus standards in the field of clinical chemistry. He emphasized the necessity for cooperative efforts among various segments of the clinical chemistry community. He cited as an example, the recent work on the characterization and production of the Bovine Serum Albumin reference materials, which have recently been issued by NBS. In this instance, the NCCLS consensus process was used to develop specifications and recommended methods for characterization of the materials. NBS followed

these consensus recommendations during the production and certification of two reference materials. These are designated by NBS/SRM 926 and NBS/SRM 927 and consist of a high purity lyophilized powder and a 7-percent solution.

Dr. Lott (ASCP) stated that two types of reference materials should be recognized. The first category would consist of primary or Certified Reference Materials, i.e., materials of the highest purity or alternatively materials with purity or composition defined by definitive methods (when available). The second category consists of "Calibration Materials" should not be considered as standards, but should be used solely for internal quality control purposes. Primary standards can be prepared by any organization, but certification should be through mechanisms such as those provided by NCCLS. Development and evaluation of reference materials should be carried out in the private sector and government agencies should not dictate what materials are to be considered primary standards. He stressed the need for many new Certified Reference Materials for RIA, NADH, hormones, proteins, and other enzymes. Priorities for the development of such reference materials should be determined in terms of current laboratory problems and medical needs.

Dr. Rand (AACC) urged the acceptance of the term "Certified Reference Material" (CRM), as defined in the AACC position paper rather than the term "Standard Reference Material, "because the word "standard" represents problems in international usage. Further, use of the word "certified" is advantageous in that it implies a formal process for the determination of purity or other properties. Acceptance of the IUPAC definition of the term "Calibration and Test Material" (CTM) was also urged. He stated that the term CRM needs better definition as it applies to the field of clinical chemistry. The need for biological matrixed CRMs was also stressed. While any organization should be able to produce reference materials, certification should be carried out by NBS. Expansion of an NCCLS advisory committee to set specifications and priorities for CRMs would be necessary. Several categories of reference materials were discussed including well-defined inorganic substances, less well-defined organic substances (e.g., bilirubin), and materials falling within the CTM class, e.g., serum-based materials. Additionally, reference materials such as Bovine

Liver (NBS/SRM 1577) and Orchard Leaves (NBS/SRM 1571) serve a useful purpose and should be supplemented with additional materials. A real need also exists for the development of a reference material consisting of an "average" human serum.

Dr. Hanson (CAP) stressed the importance of NCCLS as a consensus standards mechanism. CAP also has a direct long-term interest in laboratory standards and reference materials, as exemplified by CAP standard solutions program now in existence for 26 years. Whenever possible the CAP standard solutions are traceable to NBS-CRMs. He cited the CAP involvement in other reference material development including cyanmethemoglobin, crystalline bilirubin and the certification of these materials by CAP. Priorities should be established based on medical needs. The CAP Laboratory Survey data base would supply considerable information regarding the most important medical needs.

Dr. Carter (CDC) stated that CRMs are the cornerstone of the analytical measurement system. CDC's mission in clinical chemistry is to improve laboratory quality. Definition of CRMs based solely on the criterion of purity would limit the development of other needed materials. CRMs should be defined as the "best material available," where characterization has been exhaustive with regard to such criteria as homogeneity and stability. If reference materials contain interferences, which limit their use in widely employed laboratory methods, such limitations should be noted. The entire reference material development and production process should involve a consensus mechanism. Long-term reference materials availability must be assured. The problems associated with reference materials that cannot be characterized on an absolute basis or where exact replication (of renewals) is impossible, should be addressed.

Dr. Uriano (NBS) stated that the NBS position was that a hierarchy of reference materials and measurement methods was necessary to establish a compatible clinical measurement system on a national or international basis. He distributed two figures from a recent paper by G. A. Uriano and C. C. Gravatt published in "Critical Reviews of Analytical Chemistry" (Vol. 6, p. 361, Oct. 1977) entitled "The Role of Reference Materials and Reference Methods in Chemical

Analysis." Figures 1 and 3 in that paper describe the technical role of Certified Reference Materials (primary-reference materials) and Calibration and Test Materials (secondary reference materials) in an "idealized" national measurement system. The paper describes the relationships between various levels of methods and reference materials and also the relative roles of NBS, other government agencies, voluntary standards organizations and manufacturers in assuring that the accuracy inherent in definitive methods of chemical analysis can be transferred into general laboratory use.

The NBS position is that the degree of characterization and the intended use should determine whether a material is to be denoted as a Certified Reference Material or a Calibration and Test Material. CRMs should be characterized by definitive methods when available. They might consist of a pure material or a highly characterized analyte in a biological matrix. CRMs should only be used in critical quality control applications, for method development or evaluation, or by manufacturers in the preparation of CTMs.

Dr. Uriano emphasized that NBS/SRMs are produced and distributed in a manner that assures that SRMs do not compete with commercially produced reference materials. In contrast to CRMs, CTMs generally are less well- characterized and are intended for more routine use in field applications. Because of their widespread usage, commercially manufactured CTMs are crucial to assuring accuracy in clinical laboratories on a national basis. Good internal quality assurance programs as well as external quality assurance programs (e.g., the College of American Pathologist Clinical Laboratory Survey Program) are also needed in order to maintain a compatible measurement system over long periods of time.

The NBS position is that general technical criteria for the development of reference materials can be developed but that such general criteria must be applied in different ways to different materials. Good Manufacturing Practice is the key. Validation should be primarily through the certification or characterization process itself and not through independent (and costly) independent validation after the reference material is produced.

NBS currently uses both expert panels and formal market surveys to establish CRM priorities. It would welcome the establishment of a national consensus mechanism such as might be established by NCCLS to help develop meaningful priorities.

A series of agenda topics were discussed with the view that recommendations might result. The topics and resulting recommendations are presented in the order of their discussion. All recommendations were approved by unanimous consent of the working group members.

Topic 1: Nomenclature and Utilization

Definitions contained in the various position papers and those recommended by organizations such as ISO and IUPAC were reviewed. The working group members agreed that the definition of reference materials should be in terms of their degree of characterization and their use. Considerable discussion ensued with the following recommendations being made for both nomenclature and use.

Recommendation 1: Nomenclature

Three classes of reference materials should be designated for use in clinical chemistry. They are: (1) Certified Reference Material (CRM); (2) Calibration and Test Material (CTM); and (3) Control Material (no acronym). Only CRMs and CTMs should be considered as "standards."

Recommendation 2: Characterization and Use of Reference Materials

(a) CRMs should be characterized by definitive methods (when available) and/or exhaustive characterization by independent reliable techniques. Major use should be to assure the long-term adequacy and integrity of measurement systems, for the validation of CTMs and to aid in establishing the performance characteristics of methods.

(b) CTMs must be traceable to a CRM (if available) by a reference method or well-accepted methods. Major use

should be to help maintain calibration of individual laboratory measurement systems. Where CRMs and acceptable measurement technieques do not exist, then the term "Provisional CTM" would be appropriate. (NOTE: The term "control material" was felt to be outside the scope of the working group and was not further addressed. However, the importance of these materials to maintaining within-laboratory quality control was recognized.)

Observer Comment by Dr. Bondar:

"With reference to the question of what a reference material can or cannot be used for, I submit that declaring a material is not good for a particular application does not necessarily imply that it was tried and does not work. I would suggest that it be stated that a reference material (CRM, CTM, or Control Material) be declared acceptable for those applications for which it is qualified (with experimental verification) and for no others until it is so qualified."

This comment was discussed and the essence of the comment recognized as important. Both uses and the limitations on use of reference materials should be clearly stated by the producer.

Topic 2: Technical Criteria for Development and Certification of SRMs

Dr. Lott had previously prepared a summary of the different aspects of the technical criteria appearing in the various position papers. The ensuing discussion was facilitated by Dr. Lott's outline. The consensus of the panel members was that general technical criteria for characterization of RMs can be delineated but the specific criteria must be developed with regard to individual analytes.

Recommendation 3: General Technical Criteria for Reference Materials

The following general criteria should apply to CRMs: (1) specific composition; (2) homogeneity; (3) physical characteristics (e.g., color, particle size--importance of these will depend on specific reference material); (4) other chemical characteristics (e.g., reactivity); (5) biological characteristics (e.g., biological hazards, sterility); (6) stability; and (7) preservatives, if any.

With regard to this recommendation, several general comments are in order: (1) the term specific composition includes characterization of both analyte and matrix composition and content where end-use requires knowledge of both. Purity, except for "pure" substances, is not a sufficiently inclusive term. Statistical uncertainties of the certified data should be established and clearly stated; the uncertainty should take into account method precision and bias as well as material varability (homogeneity); (2) degree of heterogeneity should make only a small contribution to total analytical uncertainty; (3) sterility testing, where appropriate, should be carried out according to USP or other recognized procedures; (4) a minimum of 1-2 years of stability (ideally) under stated storage conditions should be guaranteed for CRMs. Further, the manufacturer should package the CRM to assure its stability and integrity; (5) methods used in characterization (including stability and homogeneity) should be stated and resulting data be made available where necessary; size of sample tested for homogeneity should be stated; (6) potential problems exist with regard to full disclosure of manufacturing processes for CRMs. The concern arises primarily from the necessity to guarantee long term availability.

Recommendation 4: NCCLS Establishes Technical Criteria

NCCLS is the appropriate body to develop general technical criteria for the characterization and certification of CRMs by a consensus process and to establish specific criteria for individual materials.

Recommendation 5: Certification of CRMs

CRMs should be certified by NBS or other qualified organizations recognized by NCCLS.

Recommendation 6: Disclosure of Proprietary Information

The issue of disclosure of proprietary information by commercial producers of CRMs should be resolved through future consideration by NCCLS; details of the process of production should be "available" for future reference.

Recommendation 7: Technical Criteria for CTMs

Similar general technical criteria as cited above should be applied to the development of CTMs. (It was recognized that in certain cases the criteria need not be as rigidly applied.)

Topic 3: Validation of CRMs and CTMs

Considerable discussion concerned different meanings of the words "validation" and "certification" and procedures for assuring the validity of data supplied with CRMs or CTMs. Although this issue was never resolved, several panel members agreed that "certification" involves the technical or laboratory process whereby the producer obtains and guarantees correct property value(s) of the reference material. In contrast "validation" implies a process whereby an organization other than the producer reviews and verifies the property value(s) claimed by the producer of the reference material. Other panel members felt that validation and "certification" of CRMs were more or less synonymous.

Recommendation 8: Validation of CRMs and CTMs

Independent validation beyond that provided by the certifying mechanism (described in previous recommendations) is not necessary for CRMs. The issue of independent validation of CTMs should be resolved by NCCLS.

In developing this recommendation, panel members expressed concern that independent validation might lead to duplication

of effort, unnecessary costs, and undue delay in production and issuance of needed reference materials.

Topic 4: Labeling

Little discussion was held concerning this topic. Total consensus was that FDA has total jurisdiction in this area.

Recommendation 9: Labeling

Both CRMs and CTMs must adhere to FDA labeling regulations. Producers of CRMs should provide information concerning the confidence limits of the values that are certified.

Recommendation 10: Use of Units

The "Systeme International d'Unites" or "International System of Units" designated as "SI" units should be used together with conventional units on all documents concerned with reference materials.

Topic 5: Availability of RMs

Many panel members expressed concern about mechanisms that might be implemented to assure long term CRM availability. Obviously, no producer can absolutely guarantee CRM availability under all circumstances. This concern led to the following recommendation:

Recommendation 11: Availability of CRMs

The certifying organization is responsible for assuring the long-term availability of CRMs. In case a previously issued CRM becomes unavailable from the producer, NCCLS should provide a mechanism for alternatives to be used in the interim period.

Topic 6: Priorities

The establishment of priorities for new CRMs, in particular, was identified as an important issue. Concern was expressed that any mechanism established take into account input from all segments of the health-care community.

Basis for establishing priorities should be dictated primarily by patient needs.

Recommendation 12: Setting of Priorities of CRMs

NCCLS should establish an expert panel to help set CRM priorities. The panel must consist of representatives from all segments of the health-care community. Priorities should be published, widely distributed, and periodically reviewed and updated.

GROUP 2.2 Biological Reference Materials

Chairman: Dr. Raymond E. Vanderlinde (AACC)

Participants:
Dr. Jane W. Neese (CDC)
Dr. Amiran Daniel (FDA)
Dr. Dennis Reeder (NBS)
Dr. Huey V. Auger (NCCLS)
Dr. Robert Rej (AACC)
Dr. Hermann Peter Lehmann (ASCP)
Dr. John G. Batsakis (CAP)
Dr. Joseph L. Giegel (Industry)

Observers:
Dr. Glenn Bartlett (American Hospital Supply)
Dr. Charles Breuer (Fisher)(NCCLS)
Dr. Gordon Guerrant (CDC)
Dr. Johannes Koedam (International)
Dr. Myron Kuchmack (CDC)
Dr. Itano Masashi (CAP)
Dr. Robert Menson (Hyland)
Dr. Max Moody (Burrows Wellcome)
Dr. Charles Reimer (CDC)
Dr. Eric Sampson (CDC)
Dr. Albert Smith (DuPont)
Dr. Henry Tomizawa (Ortho Diagnostics)
Dr. Ronald Upton (Pfizer)(NCCLS)
Mr. Voyce Whitley (FDA)
Ms. Martha Winstead (ASMT)
Dr. K. Pierre (Beckman)
Mr. Jay Smith (CDC)
Dr. John H. Reynolds (Becton, Dickinson)
Dr. Pat Yananton (Roche Diagnostics)
Dr. Richard H. Davis, Jr. (DuPont)
Dr. Jean-Pierre Bretaudiere (France)

Assignment

To outline and partially specify the criteria, selection, validation, characterizing, and labeling sample-simulating biological matrix materials.

Initial Agenda

1. Nomenclature

   - Discussion of basis of a system (purity, intended application, degree of characterization, etc., or a combinition?)

   - Applicability of proposed terms (position paper definitions, and others)

2. Uses of Biological Reference Material as part of the National Reference System (include method development, transfer of accuracy base.).

3. Discussion Topics

   - Definition by analyte such as chemically defined; multiple forms (e.g. calcium: ionized, salts, protein bound; isoenzymes); incompletely defined (total proteins).

   - Definition by the mode by which the label value is established: analysis; quantitative addition; combination (including depletion).

   - Definition of the matrix: natural and synthetic; "disease concentrations;" interference components (normal and abnormal)

   - Specifications for accuracy, purity, source, form, homogeneity, congruence, clinical simulations.

4. Adoption process for Specifications

5. Validation and Confirmation process

REPORT OF WORKING GROUP 2.2

Introduction

The Chairman (Dr. R. Vanderlinde) of the working group, following presentation of the rules of debate, decided to discuss the following agenda items in order.

1. Definitions applicable.
2. Uses and purposes of a biological reference material (BRM).
3. Technical specifications for the BRM.
4. Accreditation, certification and labeling of the BRM.
5. Consideration for establishing priorities.
6. Organizational structure within which the BRM would be integrated as part of a NRSCC.

As discussion progressed, overlap occurred on occasions, with an ebb and flow between the above agenda items. Such action mainly occurred as the participants sought to clarify how a particular point at issue would affect a given agenda item.

Organizational Positions (Summary Statement)

Prior to a detailed discussion on each agenda item, the chairman had each participant present a summary of their organizational position. Many points were in agreement, such as that reference materials are essential to a functioning NRSCC. The preparation, certification, and availability of a material such as those currently identified as an SRM by the NBS needs to be continued.

The use of a BRM to test or probe the problems of interlaboratory variability, inter-method validity and particularly to examine matrix effects upon method, had broad if not unanimous agreement. Dr. Giegel represented that such a material could be utilized as calibrators, for quality control and for proficiency testing. Dr. Rej was in partial agreement but pointed out that although these materials may also be ideally suited as calibrators and probes in external or internal Quality Control Programs, these uses are clearly secondary. Obvious disagreement on this use was expressed by Drs. Neese and Daniel. The question of uses applicable to a BRM was thoroughly debated during the development of a definition for the BRM.

The summary positions agreed that the BRM should be traceable if possible to higher level material as an SRM or verified by an appropriate reference or definitive method. Dr. Lehmann offered a detailed description as to content and

preparation of a BRM.

All summary positions agreed that the primary goal of a BRM should be directed to resolving medical needs. Additionally, there was a general tone or atmosphere supporting the consensus organizational approach to implement a NRS. The NCCLS was given as an example of an operating model.

Definitions

A. Development of Definition for CRM

Dr. Vanderlinde proposed that outlines and items presented in position papers be used as the basis for discussions, so that we would be able to come up with a report at the end of the meeting.

The debate to consider the establishment of definitions for a BRM first addressed general nomenclature. While each participant had a feel for the terms definitive, reference, CRM, SRM, calibrator, control, methods and materials, when these were intertwined with words such as use and transferability, chaos developed rather than a flow towards acceptance.

The group abandoned the above approach and elected to consider first the CRM definition offered by Dr. Rej. The characterisitics of a CRM include a highly characterized material for which the details of analysis and preparation are open and subject to review by the users. The CRM may be prepared by any person as long as guidelines and specifications adopted by the consensus organization are meticulously followed. While CRM usage would generally apply to verification and transferability of methods, particularly reference methods, a user should not be restricted from applying the CRM for multiple purposes.

Having no real problem with the above points, the group became briefly embroiled over the descriptive term "reference". Did reference mean highly purified or just represent appropriate characterization? Dr. Vanderlinde graphically outlined a scheme with different levels of materials and matrices. Any combination of the above could

represent a reference material.

The acceptance of the qualifying adjective "certified" seemed to break the confusion. The group then unanimously agreed to adopt the International Standards Organization (ISO) phraseology for a CRM as a practical working definition for the group.

Definition of CRM

A reference material accompanied by or traceable to a certificate stating the property value(s) concerned, issued by an organization which is generally accepted as technically competent (ISO).

B. Development of Definition for BVTM:

Dr. Neese suggested that since the group was having problems arriving at a general nomenclature for materials that they concentrate on defining biological reference materials, the topic for this working group. To initiate discussion, she presented a definition from an earlier CDC draft:

> "The term 'biological reference material' will be used to refer to materials composed of or simulating biological fluids or tissues as they occur in health and disease to be used for evaluating methods and other materials."

There was discussion on whether usage of the material should be included in its definition. The definition was resubmitted eliminating the last phrase, "to be used for evaluating methods and other materials." Dr. Daniel said that this was identical to the FDA definition. Dr. Lehmann proposed that a more detailed definition was desirable and presented the ASCP definition:

> "A Biological Reference Material is one consisting of one or more analytes, in their purest form available, contained in a matrix resembling, as closely as possible, the medium in which the analytes are measured in the clinical laboratory."

There was discussion as to whether this definition was too specific with respect to both the analyte and matrix. Relative to the analyte being in the purest form available, both Drs. Auger and Daniel questioned whether this would apply only to added substances and would perhaps eliminate utilization of analysis of endogenous analytes. Dr. Rej was concerned that the requirement that the matrix resemble as closely as possible the medium measured in the laboratory would limit the use of such materials as NBS orchard leaves and simulated matrices such as polyvinylpyrrolidone (PVP).

Dr. Reeder proposed the following definition:

> "A biological reference material is a material from a biological source used either as analyte, matrix or both to simulate biological tissue or fluids as they occur in health and disease."

Drs. Rej and Neese objected that this would exclude pure materials from non-biological sources in a synthetic base, such as electrolytes in PVP. Dr. Auger raised the point again of whether a definition could be developed without some agreement on use. He, Dr. Neese, and Dr. Daniel were in agreement, for example, that these materials would not be used as calibrators. Dr. Giegel questioned whether they would be used as proficiency testing samples. To this, there was general consensus that these materials would be too scarce and expensive for such usage. Dr. Auger proposed that the group attempt to derive a name and function for these materials as they relate to an analytical reference system framework. Such a system would include a high quality standard reference material which would be used to establish the benchmark for the analyte. A method would further define the benchmark. Then verification materials would be employed for evaluation and transfer of accuracy of the National Reference System. This could include their use in the development of working methods. Dr. Giegel concurred that our charge was the materials component of a National Reference System and supported Dr. Auger's approach of delineating function from which the definition would derive at the end.

Dr. Butler in returning from the meeting of Group 2.1 said he thought that group was close to a working definition

for Certified Reference Material (CRM), Calibration and Test Material (CTM), and Control Material. There was some consensus in Group 2.2 that perhaps it could settle on a working definition, but that the description of this material as a calibrator had been eliminated earlier. The FDA definition was proposed:

"Biological reference materials contain the analyte in question in a suitable matrix so as to simulate patient specimens."

Some minor changes were made in the terminology of this definition, and it was suggested that implications for use - mainly as a validation and transfer material - be incorporated in the name. The final, unanimously accepted name and definition was:

> "Biological Validation and Transfer Material - A material containing the analyte to be measured in a suitable matrix such as to simulate patient specimens."

Following discussions on the next day, a definition was further amended and accepted.

Definition of Biological Validation and Transfer Material (BVTM)

> "Biological validation and transfer material contains the analyte to be measured in a suitable matrix so as to simulate patient specimens. The physical matrix effects are important in some instances. It is therefore, important that the BVTM have physical matrix properties closely resembling the physiological fluid."

(The last two sentences were added to call attention to the importance, particularly with artificial matrices, of simulating physical properties of patient specimens such as viscosity, which can affect such methodological processes as sample measurement and aspiration).

Uses for the BVTM

Next, the participants were polled for their positions on the detailed utilization of biological validation and

transfer materials.

Dr. Reeder stated that the NBS position had been developed for a different nomenclature, i.e., primary materials to be used for transfering definitive values to reference materials and secondary calibrator materials, and that these uses may not be appropriate in light of the definition developed by the group for BVTMs.

Dr. Auger reiterated that BVTMs are for use in the validation and transfer of accuracy and suggested that they would perhaps be restricted to utilization in a reference system.

Dr. Batsakis proposed that use be confined to a reference system for analytes in which long-term data indicated a need for such materials.

Dr. Daniel felt that few people would ever set up reference methods and that would limit use. His position was that BVTMs primarily be used for interrelating definitive, reference, and routine laboratory methods, thus for defining bias, or essentially the same as the AACC group stated in their position paper:

> "Biological matrix reference materials (now BVTM) are intended for use as probes in the assessment of the reliability and performance of an analytical process. They shall be used to interrelate definitive, reference and routine methodologies and help define analytical biases associated with established methods."

There was considerable interaction on this proposal resulting in its not being accepted.

Dr. Neese suggested that there are two categories of utilization. The main use is for definitive and reference methods, to establish and maintain the accuracy base; the second is to transfer that accuracy base by using BVTMs for such purposes as evaluation of other methods, testing diagnostic products, and transfering benchmarks to other users' materials. After some discussion, the following was unanimously accepted:

Major Uses of the BVTM

A. Methodology Development, Validation, and Maintenance

1. Assess accuracy during the development of definitive and reference methods.

2. Compare definitive and candidate reference methods.

3. Test interlaboratory transferability of candidate reference methods.

4. Assess or assure stability of definitive and reference methods with time.

B. Transfer of Accuracy Base

1. Assist in evaluation of other methods or materials.

2. Assist in evaluation of diagnostic products.

3. Aid in the development of new routine methodologies.

There was discussion on whether a statement should be added that the above are not the only uses. It was concluded that that was implicit in the prefix "Major". Besides, it would be essentially impossible to prevent these materials from being used for other purposes.

Guidelines for Specifications of BVTM

Following the adoption of uses for a BVTM, the chairman asked the group to consider the technical specifications of a BVTM. Dr. Auger vigorously recommended that in the preliminary design proposal for a BVTM, the proponents must consider and apply contraints entitled:

1. Analytical goals.

2. Physical and chemical properties of analyte.

3. Reference method.

4. Cost vs Benefit.

Dr. Daniel supported the concept, reminding the participants for example, that if the designer did not heed the analytical goal constraint, one might set precision levels so tight as to make the material nonapplicable. There were additional comments supporting the remaining design constraints. The characteristics of the analyte might require a particular matrix. The methods available to validate and certify the BVTM may have limitations with respect to specificity, interference, etc. and the cost may be so prohibitive as to limit use. The adopted form did undergo mild alteration (for consistency) during the final review period. The group unanimously adopted the following design criteria constraints:

Anlytical Goals - The desired level of accuracy and precision will be based on patient care and needs. These goals will determine the need for the BVTM for each analyte and also provide a basis for the establishment of priorities.

Physical and Chemical Properties of Analyte - Properties, such as stability and reactivity, are major factors in deciding both the final matrix and the storage and handling conditions for the BVTM.

Method - The limitations of any evaluating procedure must be considered when designing the BVTM.

Cost vs Benefit - In principle, the ideal BVTM would be a liquid material identical with the clinical sample that could be stored at room temperature for an indefinite period of time. In practice, however, the incorporation of all these features into the BVTM is not feasible. Therefore, during design and development, a continued assessment of cost versus benefit for those features relating to performance, stability and handling will be essential.

Composition of the BVTM

As an initiating step for discussion, the group considered Dr. Lehmann's detailed description on the composition of a BVTM. His outline detailed characteristics of both analyte and matrix and cited complex examples.

With a minimum of discussion (basically considered

editorial content), the group adopted definitions for an analyte and matrix.

Definition of an Analyte

The analyte is the component to be measured. The (analyte) component in the BVTM should, as far as possible, be identical in composition and structure to the component in the physiological system.

Definition of Matrix

The matrix is the milieu in which the analyte is contained and consists of all other components of the material, including other analytes not being assayed by the specific procedure.

Dr. Lehmann's original proposal that the medium be as free as possible from all compounds which may interfere with the reference method was discussed at length.

Dr. Auger, who at first recommended inclusion of known substances, then acknowledged and was supported by Dr. Daniel that if all known and potential interferences must be added, then the required number of BVTM would become very large. Dr. Gruber (observer) believed that normal interferences are important and should be present. Dr. Lehmann suggested that the identification of interferences applies to the reference method (the validating or accreditation system). The group agreeed with Dr. Neese's compromise proposal: BVTMs simulating normal and abnormal patient specimens are desirable, as well as specimens with and without known interferences.

Having hurdled the problem of interferences, the group faced the need to establish guidelines for the mixing of analytes and matrices forming a BVTM. While appreciating the detail supplied by Dr. Lehmann but reminded of Dr. Butler's (coordinator) charge that the group was to develop a guideline for potential users, Dr. Vanderlinde's scheme was reconsidered. That proposed system lists varying levels for analytes and matrices and further permits any combination as dictated by intended use. The final guideline for characterization or composition of the BVTM follows.

Characterization of BVTM

Biological validation and transfer materials (BVTM) simulating normal and abnormal patient specimens are desirable, as well as specimens with and without known interferences. Known or potential interfering substances present in the BVTM affecting the reference method must be specified qualitatively and quantitatively.

The desired composition of the BVTM will depend upon its intended use. The purity of the analyte will depend upon the type of material assayed. For each BVTM a decision will have to be made whether the preparation may involve addition of analyte of certified purity to a matrix, or purification of the compound within a matrix, or both.

The following scheme is proposed for the combination of analytes and matrices into a BVTM:

Analyte		Matrix	
α	Highest purity	δ	Defined e.g., H_2O, buffer.
β	High purity with the impurities defined	ϵ	Partially characterized, e.g., albumin, polyvinylpyrrolidone.
λ	Partially characterized: Impure but reactivity or activity known.	ζ	Poorly characterized matrix, e.g., serum, urine, cerebral spinal fluid.

Preparation of a BVTM

The group believed that its charge was to develop a guideline of factors that minimally should be considered when preparing a BVTM. In that light those technical considerations necessary for preparation of a BVTM are described below.

One should appreciate that the discussion surrounding technical specifications resulted in corrections or

clarification of other items such as the definition and composition of the BVTM.

A. Technical Considerations

1. Accuracy of assigned values with confidence limits.

2. Definition of base and additive.

In all cases, despite the variable origin of the component materials, the composition of the material should be known as completely as possible. Sources and nature of components and any modifications should be specified. Information on additives, antibiotics, preservatives, etc. must be stated even if the effects on analyses are unknown or presumed to be negligible.

3. Physical form (liquid or lyophilized).

4. Stability.

5. Homogeneity.

6. Capability to simulate clinical specimens.

7. Hazards.

As an example of typical detailed requirements, referral is made to the NCCLS document on calibrators (ASC-2 or its revised version).

A formulation protocol for the preparation of each BVTM should be developed in advance. The assignment of target values, the types of validation necessary and testing for stability and homogeneity will be part of the formulation protocol which shall be submitted to peer review (the consensus mechanism). The final accreditation and certification shall be through an NCCLS like consensus mechanism.

An important final phase in preparation of the BVTM is the proper labeling. The group believed that the current FDA requirements may well be all inclusive. To assure that nothing would be left to chance the following list of

labeling requirements prepared by Dr. Lehmann was recommended as a minimum requirement:

B. Labeling

Labeling and certification information should meet all FDA requirements and shall include, if not otherwise specified:

a. Name(s) of analyte(s).

b. Nature and source of matrix (e.g., human serum, human albumin, bovine albumin, urine, etc.).

c. Concentration with confidence limits of analyte(s) in the material.

d. Preparative procedures - purification steps for matrix, names and purity of analyte(s) (sources) added, etc.

e. Identity and specifications of all additives (stabilizers, antibiotics, etc.) present.

f. Identity and maximum levels of compounds present that are known to be possible interferences with reference procedure.

g. Reference procedure(s) used to determine concentration of analyte(s).

h. Definitive procedure, if any, used to determine analyte concentrations.

i. Names of evaluating laboratories and director.

j. Stated, or referenced, statistical procedures used to determine analyte concentrations.

k. Decay characteristics of analyte(s).

l. Manufacturing date.

m. Date of analysis by evaluating laboratories.

n. Storage requirements.

o. Stability and tolerance limits (shelf life).

p. Manufacturer.

q. Name and address of person responsible for BVTM certification.

Priorities

No recommendation or mechanism for the establishment of priorities was made. That role was referred to the analytical goals groups.

Organization

The working group and observers agreed on the necessity for an open National Reference System in Clinical Chemistry to which any national or international group or organization may make submission of a candidate material or method. The group believed that the NCCLS had a structure from which an operational NRS could develop.

The working group reviewed their recommendations. The chairman thanked the participants and observers for their sincere efforts. The session then adjourned.

TOPIC 3. The Methods Development Component of a National Reference System

Topic Coordinator: Mr. Gerald E. Gallwas
(Industry) Orientation, Procedural Instructions, and Guidelines

Group 3.1 Definitive Methods

Chairman: Dr. Robert Schaffer (NBS)

Participants: Dr. John Liddle (CDC)
Dr. Charles Furfine (FDA)
Dr. Matthew M. Patton (NCCLS)
Dr. Horace Martin (AACC)
Dr. Robert C. Rock (ASCP)
Dr. John B. Fuller (CAP)
Mr. Walter Slavin (Industry)

Observers: Dr. Eleanor Berman (AACC)
Col. William Cowan (AFIP)
Mr. George Lyna (NCCLS)(I.L.)

Assignment

To outline and partially specify the criteria for selection, validation, and adoption of definitive methods as well as how they will be applied.

Initial Agenda

1. Definitions and Questions

   A. How can analyte concentration be defined "unambiguously" by an analytical procedure?

   B. What is the "end-purpose of" a definitive method?

   C. Comparison of definitions proposed in position papers

2. Definitive method techniques - criteria for and examples of

3. Definitive Method Application

4. Relationship to Reference Methods, Provisional Method Principles and Reference Materials

5. Other uses

6. Procedure for Selection, Validation, and Adoption of Definitive Methods

   A. Peer Review

   B. Performance Specification

   C. Independent Validation

   D. Resolution of Discrepancies

   E. Adoption Procedures

7. Procedure for Comparing Reference methods with Definitive Methods

8. Procedure for Examination of Reference Materials using Definitive Method

REPORT OF WORKING GROUP 3.1

Opening statements by the delegates were essentially affirmations of the written positions previously presented by their organizations. Hence, the meeting began with agreed recognition that analyses by a definitive method would give analytical values that are like "true values", and that reference methods and, in turn, working laboratory methods could be evaluated for accuracy by comparing their results against those obtained by a definitive method. However, the definition of a definitive method remained controversial, and that item was deferred to the end of the agenda with the thought that the working group's discussion would provide added opportunity for sharing ideas on the nature of a definitive method before attempting a definition to recommend to the Conference.

Analytical methods that might qualify as definitive were discussed at length. It was recognized that emphasis must be placed on both the scientific soundness of analytical

principles used as well as the data obtained. Dr. Liddle emphasized the former and Dr. Schaffer the latter. Much attention was given to isotope dilution-mass spectrometry as a definitive method but a number of other methods were considered as well; e.g., gas liquid chromatography, high pressure liquid chromatography, flame photometry. Such techniques might be appropriate if the necessary study and documentation could be provided to ensure the accuracy attained by their use. The interest expressed in the use of isotope dilution-mass spectrometry for definitive analysis was seen to be due to its two-fold property of separating the analyte from the matrix (for matrix-free measurement) and measuring the ratios of the isotopic internal standard and nonisotopic analyte. It was noted that the mere application of a method which may have the potential for providing definitive analyses, such as isotope dilution-mass spectrometry, would not ensure that definitive analytical values were obtained - that is to say, the accuracy of the analysis would depend on how the method was applied.

The working group considered the performance characteristics expected of a definitive method. The necessity for appropriate chemical specificity and chemical sensitivity were discussed (the latter with respect to both detection limit and ability to discriminate between differences in concentration). Dr. Fuller emphasized that the method should necessarily be appropriate for use on patient specimens with respect to both range of concentration and matrix. [Chairman's added note: Rather than 'patient specimens' the statement might better read 'patient-like specimens'.]

The need to set maximum acceptable limits to the uncertainity of the results obtained from the definitive method was recognized. This uncertainity was expressed in terms of the systematic and random error estimated by statistical analysis. However, there might be more than one method for obtaining definitive analytical results for the same analyte, and unless their mean values were identical, the overall uncertainty would be greater than that derived from the initial method alone. If a systematic difference were found, that is, if the difference in their mean values exceeded tolerable limits, neither method could be recognized as definitive until the difference was resolved. [See

minority report by Dr. Liddle]

Mr. Slavin addressed the need for confirming definitive method results, expressing in this way the fundamental idea that all elements of the reference system should be tested. The necessity for this confirmation was put into the form of a recommendation by Dr. Martin, seconded by Mr. Slavin and carried unanimously:

> Given a definitive method that has not yet been confirmed, one can maintain its relationship to a reference method but there is an obligation to complete the confirmation of the definitive method in another laboratory as soon as possible.

The timeliness of this confirmation would be particularly urgent when a bias is found between the definitive and reference methods; but even in the absence of such a bias, the necessity for confirmation is recognized.

In discussing the nature of this confirmation, it was recognized that it may not be possible to have exactly the same definitive method run in another laboratory that undertakes to confirm the definitive method. In fact, a significantly different definitive method might be utilized. However, given that analyses by definitive methods provide accurate results, one would expect results having a negligible difference between them. Increased assurance of the basic accuracy of the definitive method should result if a different method were used for the confirmation.

The working group discussed the role of the definitive method in a national reference system and considered possible reference systems. However, the system primarily considered was one in which a national council, in recognition of a need of the clinical community, assigns a committee to investigate how to fulfill that need in terms that include definitive and reference methods, and in that context recognizes the special role of the definitive method in providing the basis for accurate measurement for that system.

The working group also considered some of the aspects involved in the development of a definitive method. As a necessity the working group recognized that the laboratory

undertaking to develop a definitive method should submit its plans to the overseeing committee for review, with the objective of seeking as much information and guidance as possible from the community for directing that effort appropriately. Necessarily the scope of the planned definitive method should be carefully delineated, and when the development of the definitive method is complete, the results obtained together with supporting evidence for its validity are to be provided to the overseeing committee for review. The necessity for maintaining accepted definitive methods was also discussed and the working group recognized the need for having the laboratory that undertakes to set up definitive methods maintain them for the necessary national reference system uses.

In the application of a definitive method for establishing the accuracy of a reference method, the working group recognized that the scope of that undertaking must be clearly delineated, and that the nature of samples used in comparing the two methods be given particular attention. The committee should, for example, consider whether to include interferences in the samples to be analyzed. Clear goals must be set for accepting a candidate reference method and maximum imprecision. The allowed uncertainity in the reference method must be related to patient care needs as met through utilization of working laboratory methods.

In considering the results from an intercomparison of the definitive and candidate reference methods, the overseeing committee will have little problem if the results show acceptable precision and no significant bias, or bias within the preconsidered allowable maximum. However, if the bias is in excess of the preconceived goal, the committee might deal with that problem in one or two ways. It may request a continuation of the evaluation of the candidate reference method with the same or new goals or it may decide that it is desirable and necessary to accept the results but alter its original goals. As with all decisions the overseeing committee would report their rationale to the community.

Another problem that will occur when there is a significant although acceptable bias between the reference method and definitive method is whether to use the definitive

method or the reference method as the basis of the national reference method system. Should the bias between the reference method and definitive method be added to the bias that may be found between the reference method and a working method? The consensus of the working group was to defer using the definitive method base for the present. (A formal vote was not taken). Dr. Schaffer did not agree. He recognized the seeming practicality of that choice, but was more concerned for the consequence: the inevitable need to change to the definitive method basis for relating methodological inaccuracy.

In a discussion of the question of equivalency of methods, it was recognized that equivalency should have no special effect on the overall system. Thus a method that is found to be equivalent ot a definitive method should be recognized as such. Simiarly, methods equivalent to reference methods may also enter the national reference system. The question of a definitive method and a reference method being equivalent was also considered. The working group recognized that under that circumstance the necessity for maintaining both the definitive method and reference method in the national reference system would be diminished; however, having both would be beneficial for the scientific assurance demanded of the national reference system. Terming one definitive and the other reference might remain significant only from the historic view.

On discussing the definition of the term "definitive method", two basic definitions were considered. One was the definition given in the glossary of the AACC position paper;

> "An analytical method that is capable of providing highest accuracy among all methods for determining that analyte, and of sufficient accuracy for the stated end purposes of the definitive method".

The other was the IFCC definition:

> "An analytical method that after exhaustive evaluation is found to have no known source of inaccuracy or ambiguity".

A motion was made by Dr. Fuller for the acceptance of the

former definition. The motion, seconded by Dr. Rock, was accepted by the working group by a vote of 6 to 1 with Dr. Liddle casting the dissenting vote and Dr. Patton absent for the vote. Dr. Liddle indicated that a minority report would be prepared on this dissention.

Note by Dr. Liddle

I did not agree with this concept of definitive method bias during our discussion at the meeting and after careful consideration since then, my position has not changed. First, you cannot have two methods which give results with a measurable bias between them and both methods still be classified as definitive in nature. When using either of the two proposed definitions of a definitive method, only one of these methods could possibly be a true definitive method. Secondly, you cannot measure the bias of a definitive value to the theoretical "true value" for a given analyte in a biological matrix using in vitro spiked material because the material would become the accuracy base for the measuring system and not the definitive method. These ideas will be discussed in some detail in the minority report but some indication of this difference of opinion should be taken into consideration in this part of the summary report.

Additional Comment (CAP)

The College of American Pathologists would like to submit the following additional comment to the report of the study group on definitive methods:

> In view of the cost and complexity of performing definitive analyses the CAP recommends that whenever definitive analyses are performed an attempt should be made to utilize aliquots from a large pool of material for the analyses. Further aliquots of material should be stored and an inventory of materials with definitive values created. Samples from the inventory would be available to investigators undertaking projects for which such materials would be appropriate.
>
> The CAP and Hyland Laboratories will store the remaining samples of lyophilized serum from the study performed in 1975 comparing NBS definitive values and CAP survey

participant values. The concentration of calcium, iron, lithium, magnesium, sodium and potassium were determined on these samples by definitive methods at the National Bureau of Standards.

Samples of this material will be provided to investigators working on development of new methods or substantial modifications of established methods.

Requests for material should be forwarded to NCCLS, NBS or CAP.

MINORITY STATEMENT (Dr. Liddle - CDC)

1.0 Definition

1.1 Introduction

The definitive method represents the accuracy base for the National Reference System for a defined analyte in a specific matrix. Careful employment of this methodology will give analytical values (definitive values) that are the closest obtainable approximation of theoretical "true values." As such, the development and use of definitive methods are very idealistic but still highly functional concepts. The word definitive has been defined as: serving to provide a final solution; authoritative and apparently exhaustive; serving to define or specify precisely; fully differentiated or developed (1). This definition fully describes the ultimate purpose of definitive methods in the National Reference System and it is a combination of these phrases that form the basis of the published IFCC definition. The Center for Disease Control believes these concepts must be included in any statement defining the characteristics of these analytical methodologies. Methodology that contains known, but undefinable, inaccuracies or other uncertainities must not be accepted as definitive in ascribing numerical values to selected chemical or physical characteristics of an analyte. It is recognized and accepted that the adoption of these basic concepts will result in the inability to develop true definitive methodologies for poorly definable analytes. But these limitations are necessary to ensure the scientific basis of the National Reference System.

1.2 AACC definition versus IFCC definition.

The majority of members of the Definitive Method Working

Group viewed the AACC definition as a more practical, functional, working statement, while the IFCC definition was too idealistic in nature, describing characteristics unobtainable in the analytical laboratory. However, a close study of the two definitions discounts this conclusion.

The AACC definition states that a definitive method "is capable of providing highest accuracy among all methods for determining the analyte." The basic question related to this criteria is how does one fulfill this requirement without an extensive and complete (exhaustive) study of all methods? A study of this type would be an endless process as new methods or modifications of old ones are continuously being developed. A reasonable approach would be to carefully select general techniques, based on sound proven analytical principles, such as IDMS, extensively study these procedures defining their characteristics in minute detail establishing its unquestionable accuracy and then utilize these basic analytical techniques for the analysis of a wide spectra of analytes, and matrices.

The IFCC definition is a functional statement that describes the process by which a carefully selected method is elevated to the level of "definitive." The term "exhaustive evaluation" does not constitute an endless analytical process. The work required to meet this criteria can be clearly defined by the clinical chemistry community using the consensus mechanism through the NCCLS. If a method is truly definitive in nature, any source of inaccuracy (bias) or ambiguity (not to be confused with imprecision) defined during the extensive evaluation must be eliminated by accurate mathematical compensation or modification of the basic chemistry or the procedure used in the analysis. Any deviation of a given analytical result from the true value must come from pure random analytical error.

The phrase "of sufficient accuracy for the stated end purposes" in the AACC definition is an indeterminant statement because it contains an undefined term "end purposes." The defining of this term, end purposes, is critical to any discussion of this definition of the definitive method. CDC believes that sole end purpose of the definitive method is to establish the accepted true value or bench mark for an analyte in a given sample matrix. The

accuracy and precision requirements of this method should be based solely on analytical factors. Clinical and medical requirements, which continually change, should not be used to define the required accuracy and precision characteristics of the definitive methods. This concept does not involve the accuracy requirements of the reference or working (routine) methods when describing the characteristics of a definitive method. The accepted reference method for a given analyte is the bridge between the nonmedically oriented definitive method and the working methods used to generate patient data for the practicing physician.

2.0 Bias

The bias of a definitive value to the theoretical true value for a given analyte in a biological matrix cannot be measured using in vitro prepared reference materials. If this was not true, the in vitro spiked material would become the accuracy base for the National Reference System and not the definitive method. Within the National Reference System, the definitive method, by definition, is the ultimate measurement of accuracy.

Using either of the two proposed definitions of a definitive method you cannot have two methods which give results with a measurable bias between them and both methods still be classified as definitive in nature. Only one of the methods can be the most accurate or gives the closest approximation to the theoretical true value.

3.0 Summary

CDC recommends the adoption of a definition for the definitive method that incorporates the basic concept of ultimate accuracy with no known systematic errors or other uncertainities.

GROUP 3.2 Reference Methods

Chairman: Dr. Basil Doumas (AACC)

Participants:
Dr. Carl A. Burtis (CDC)
Dr. Barbara Tejeda (FDA)
Dr. Rance Velapoldi (NBS)
Col. Edward C. Knoblock (NCCLS)
Dr. Graham Widdowson (AACC)
Dr. Bradley E. Copeland (ASCP)
Dr. Noel Lawson (CAP)
Dr. Noel C. Allain (Industry)

Observers:
Dr. Paige Besch (AAC)
Dr. Henry Curme (Eastman Kodak)
Mr. Low Caprioglio (Union Carbide)
Mr. Jack Dickinson (Harleco)
Dr. Bill Dierksheide (FDA)
Ms. Pat Duncan (CDC)
Dr. Russell Eilers (CAP)
Dr. George Evanega (Bio-Dynamics)
Dr. Carl Garber (AACC)
Mr. Tom Hearne (CDC)
Dr. Martin King (Sclavo Inc.)
Dr. Victoria Leitz (E.N.I.)
Dr. Henry Nipper (AACC)
Mr. Alan Posner (Technicon)
Mr. George Sims (NCCLS)(Hycel)
Mr. Wyley Williamson (FDA)
Mr. Robert Wurzel (NML)
Dr. Stanley S. Brown (U.K.)
Dr. Jack Mogel (Sclavo, Inc.)
Dr. Robert C. Paule (NBS)
Dr. R. Ira Porterfield (Hyland)

Assignment

To outline and partially specify the criteria for selection, validation, and adoption of reference methods. This discussion will include reference methods with and without a definitive accuracy base.

Initial Agenda

1. Technical Philosophy for Development and Validation of Reference Methods

   A. Steps Required

   B. Adoption procedure

   C. Relationship to definitive methods and definitive reference materials (SRMs)

   D. Categories of analyte models

   E. Analytes with a material based accuracy definition (e.g. electrolytes)

   F. Analytes with an assigned (*per fiat*) accuracy definition (e.g. enzymes)

   G. Analytes such as drugs and drug metabolites

   H. Other accuracy problems

2. Use of Reference Methods

3. Comparison of viewpoints in position papers

4. Candidate Reference Method Selection

   A. Criteria of Performance

   B. Criteria related to availability

   C. Procedure of selection

5. Validation Procedure

   A. Steps required

   B. Materials required

   C. Blank definition

D. Performance specifications

E. Procedure for review and acceptance

F. Comparisons with definitive methods

G. Other validation criteria

H. Transferability criteria and review and acceptance

REPORT OF WORKING GROUP 3.2

The Session was called to order by Chairman Basil T. Doumas.

After a few opening remarks by the chairman, each participant was asked to give a summary of the position of his organization with regard to Reference Methods. The summaries were representative of the position papers submitted by the participating organizations.

The Working Group then moved to the discussion of the items of the Agenda.

NEED FOR REFERENCE METHODS.

It was agreed that development of Reference Methods is needed when inaccuracies in methodology have been shown to exist or if such inaccuracies may impair the optimum delivery of medical care.

It was felt that analytical accuracy is important in every measurement from a scientific point of view and also from the point of view of medical practice as it relates to patient care.

DEFINITION OF REFERENCE METHOD AS A CONCEPT

After long deliberation, the following definition of a Reference Method was developed by the Group:

"A reference method is an analytical method with thoroughly documented accuracy, precision, and low susceptibility to interferences. The accuracy and precision shall be

demonstrated by direct comparison with the definitive method and SRM or, where not available, with other well-characterized and documented analytical approach(es)."

It was moved and seconded that this definition be adopted by the Group. The motion was carried unanimously. The poll of the observers was: For - 12, against - 0, abstaining - 3.

USES OF REFERENCE METHODS

Potential uses of reference methods were discussed. The Group felt that Reference Methods could be used for the following purposes:

1. To determine analyte concentration in calibrators.

2. To evaluate bias in routine methods.

3. To test for interferences in routine methods.

4. To assess product compliance.

5. To analyze patient samples in specific clinical studies.

It was moved and seconded that these uses be recommended. The motion carried unanimously. The poll of the observers was: For - 13, against - 1, abstaining - 0.

Dr. Copeland moved to include an additional use of Reference Methods as follows:

"To establish bias relationships between human sera and other calibrator matrices."

The motion was seconded but defeated on the following vote:

Burtis	- Against	Copeland	- For
Allain	- Against	Knoblock	- For
Tejeda	- Against	Lawson	- For
Widdowson	- Against	Velapoldi	- For

Dr. Doumas voted Against to break the tie. The poll of the observers was: For - 1, against - 11, abstaining - 2.

MECHANISM FOR ESTABLISHING PRIORITIES

The Group discussed the need for setting priorities in the development of Reference Methods for specific analytes.

The following recommendation was proposed:

"A panel of representative experts shall set priorities for the development of Reference Methods. This panel will include medical practitioners, laboratory scientists, and representatives of industry and government."

It was moved and seconded that this recommendation be adopted. The motion carried unanimously. The poll of the observers was: For - 14, against - 0, abstaining - 0.

The Group also recommended that priorities for selecting Reference Methods be based on a need for such methods as evidenced by demonstrated inaccuracy in clinical practice and that such inaccuracy may impair the optimum delivery of medical care.

RESPONSIBILITY FOR THE DEVELOPMENT OF REFERENCE METHODS

The Group discussed the question: "Who should be charged with the responsibility for developing Reference Methods?" The Group felt that no one organization should be burdened with the primary responsibility for developing Reference Methods. The Group recommended that:

"Any competent analyst may submit an analytical method for consideration as a potential Reference Method to the appropriate channels of the National Reference System."

It was moved and seconded to accept this recommendation. The vote of the participants was:

Burtis	- For	Tejeda	- For
Allain	- For	Velapoldi	- For
Knoblock	- For	Widdowson	- For
Lawson	- For	Copeland	- Against

The motion carried. Poll of observers was: For - 13, Against - 0, Abstaining - 1.

STEPS IN DEVELOPMENT OF A REFERENCE METHOD

The Group recommended that the following steps should be considered in the development of a reference method:

1. Selection of the analyte on the basis of priority.
2. Definition of the analyte, where this is unclear.
3. Specification of performance requirements.
4. Selection of candidate Reference Methods or models.
5. Development and validation of the selected candidate method.
6. Tests of transferability.
7. Acceptance as a Reference Method.
8. Propagation and maintenance of the method.
9. Periodic review and revision.

During the discussion of the above points, the following additional recommendations were made: In the selection process (item 4), Dr. Copeland proposed the following:

"When a Reference Method study is being conducted by individuals, groups or conferences, consideration should be given to the relationship of new Reference Methods with previously recognized Reference Methods."

It was moved and seconded to recommended this proposal. The motion carried unanimously. The Poll of the observers was: For - 14, Against - 0, Abstaining - 0.

With regard to development and validation (item 5), Dr. Lawson proposed and the group agreed that those planning on doing interference testing should consider the following problem areas:

1. Common drugs.

2. Drugs likely to be used in conjunction with the disease state for which the test is ordered.

3. Drugs known to interfere with an already utilized method.

4. Analytic properties of the method itself which lend themselves to interference.

5. Intrinsic chemical analytes in the matrix.

6. If analyte is a drug, other drugs and metabolites of drugs which may be used in treatment along with the analyte drug.

With regard to the maintenance of Reference Methods (item 8): Dr. Lawson proposed the following:

"Maintenance of Reference Methods should include periodic analysis for the analyte in a stable pool of matrix material appropriate to the particular analyte and body fluid or tissue. The source of material, sponsoring organization, and repository for this material will be decided on the basis of needs and appropriateness of the sponsoring organization, and repository will be determined by the committee of scientists developing a Reference Method under the aegis of the National Reference System."

It was moved and seconded to recommend this proposal. The motion carried unanimously. The poll of the observers was: For - 14, against - 0, abstaining - 0.

COMMUNICATIONS AND INTERRELATIONSHIPS
AMONG WORKING GROUPS

The Group agreed that communication will play an important role in the development of Reference Methods. Dr. Eilers proposed the following statement:

"The coordinating body shall develop and provide a communication mechanism to inform, on a periodic basis, the "public(s)" involved, about the status of Reference Methods and Reference Materials being developed, the scientists involved and help that may be needed."

Dr. Lawson sponsored this proposal and it was moved and seconded to recommend this proposal. The motion carried unanimously.

The Group also recognized the desirability of fostering and maintaining communication and interrelationships between groups working in reference methodology. Dr. Eilers proposed the following statement:

"The coordinating body shall be charged to develop further international communication so that the United States National Reference System output is shared to effect the acceptance of National Reference System output on the international level."

Dr. Lawson sponsored this proposal and it was moved and seconded to recommend this proposal. The motion was carried unanimously.

RECOMMENDATIONS FOR THE RECORD

The Group also considered proposals in other areas pertaining to Reference Methods.

Dr. Velapoldi proposed:

"It is evident that the major portion of the organizational component required to establish a National Reference System already exists in NCCLS. We therefore recommend that NCCLS evolve into this council or body."

It was moved and seconded to recommend this proposal. The vote was:

Allain	- For	Tejeda	- For
Copeland	- For	Widdowson	- For
Knoblock	- For	Velapoldi	- Abstaining
Lawson	- For	Burtis	- Against

The poll of observers was: For - 14, Against - 0, Abstaining - 2.

Dr. Copeland proposed that:

"The Conference recognizes that the medical and clinical investigation community of the United States has had de facto agreements since 1930 concerning "Reference Methods" for many of the analytes of medical importance. (see the ASCP position paper.)" It was moved and seconded to recommend this proposal. The motion carried unanimously. The poll of the observers was: For - 0, Against - 3, Abstain - 10.

Col. Knoblock entered a comment for the record as follows:

"A framework for method validation by the consensus approach currently exists with NCCLS. Evaluation, transferability, publication and dissemination may be accomplished by this mechanism at minimum expense to the National System."

The Chairman thanked the participants and observers for cooperation and input.

GROUP 3.3 Provisional Reference Principles

Chairman: Dr. Ronald H. Laessig (NCCLS)

Participants:
Dr. Dayton T. Miller (CDC)
Mr. Alfred Bracey (FDA)
Dr. David M. Jeffers (NCCLS)
Dr. Al Free (NCCLS)
Dr. Thomas O. Tiffany (ACC)
Dr. Irwin Schoen (ASCP)
Dr. Charles G. Massion (CAP)
Dr. Andris Indriksons (Industry)

Observers:
Mr. John Borris (Fisher Scientific)
Mr. William Damaska (FDA)
Ms. Jan Donelson (PMA)
Dr. Carl Henson (Coulter)
Dr. Ira Porterfield (Hyland)
Mr. Edward Rice (Smith-Kline)

Assignment

To outline and partially specify the criteria for selection of analytical principles or materials for interim use as common bases of reference.

Initial Agenda

1. Definition of Provisional Reference Principles based on

   A. A method
   B. An analytical approach
   C. A material

2. Application

   A. Role in the National Reference System
   B. Other uses
   C. Guidelines to their application
   D. Potential problems

3. Selection of Principles for

   A. A method
   B. An analytical approach

C. A material
D. Documentation and review

4. Selection Process

A. Steps
B. Non-selection implications

5. Validation

A. Extent required
B. Independent sources?

6. Documentation and Review

A. Nature and Amount
B. Literature Source Confirmation

7. Transferability

A. Criteria
B. Requirement?
C. Process for

8. Formal Adoption

A. Flexibility
B. Updating
C. Disadvantages vs. Advantages

9. Information Systems

A. Repository
B. Transfer system

REPORT OF WORKING GROUP 3.3

Historical Perspective

During the early work of the FDA's In Vitro Diagnostic Advisory Committee on In Vitro diagnostic products, in which the CDC, the scientific community, and NBS were prominent participants, it became apparent that there was a national need for highly reliable, fully defined and tested reference methods. This need has led to a considerable effort by the scientific community, particularly CDC, in the development of a glucose reference method. At the same time, it became apparent that the development of reference methods is necessarily an arduous and slow process. Consequently, for the immediate future it is unlikely that there will be fully validated and tested reference methods available for many analytes.

As an interim approach, the concept of using methodological principles (MP) was proposed. In this context, the MP was to be a simple statement of an analytical approach (i.e. atomic absorption) and possibly a number of imposed constraints or conditions (i.e. sample preparation, reagent specifications, or calibration procedures), which were considered to be essential to achieve uniformity of analytical results. After a period of discussion in the scientific community, the FDA contracted in July 1976 to have the NCCLS undertake an investigation of the practicality of this approach. The NCCLS convened a panel of experts whose objective was to assess the degree to which agreement could be reached on a MP for each of 18 common analytes. This work resulted in the publication, in July, 1977, of a proposed standard: "PSC-5, Methodological Principles for Establishing Principal Assigned Values to Calibrators."

The concept of using MPs as reference points was further defined by this Conference in November, 1977. Under the broad topic of Methodological Components, three separate working areas were recognized: Definitive Methods, Reference Methods and Provisional Reference Principles. In the deliberations of the latter Working Group, as will be seen, a distinction was drawn between the related terms "methodologic principle" and "provisional reference principle."

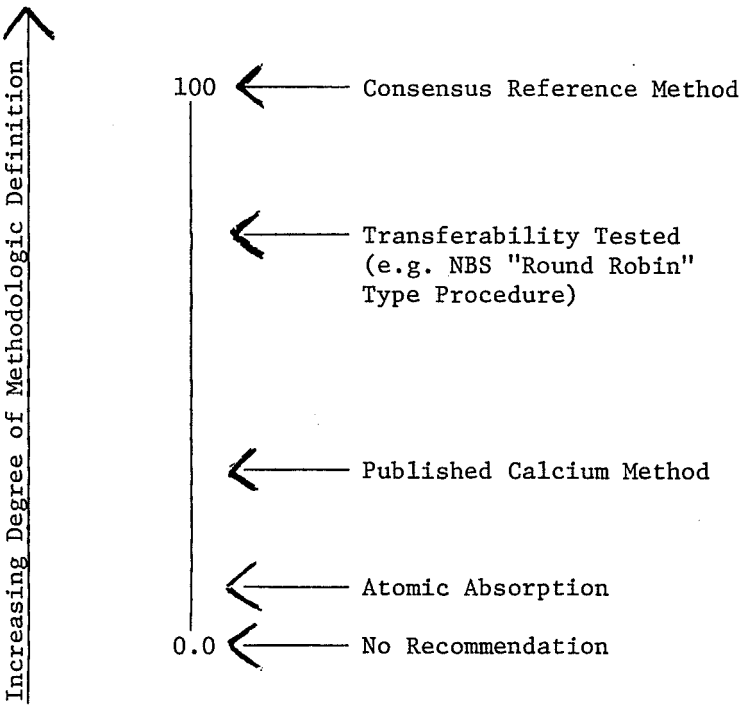

Figure 1. Scale of potential MPs.

The Need for Definition

The MP working group considered a twofold problem related to identity. (a) Among the seven working papers there was an obvious lack of uniformity in the concept, definition, terminology and even in the recognition of a need for MPs. Several position papers completely neglected to discuss the concept; one sponsoring organization did not appoint a representative to the Working Group. (b) The organization of the Conference recognized three specific method-related concepts: definitive methods, reference methods, and provisional reference principles; this might be interpreted as charging this Working Group with considering all areas in methodology not encompassed by the first two. Consequently, it seemed necessary to have the working group discuss a wide spectrum of potential definitions or components of definitions before limiting itself to a specific concept of MPs and developing a suitable (final) definition.

Conceptual Bases of Methodologic Principles

The main divisive element encountered in developing the concept and applications of MPs relates directly to the degree of specificity embodied in the statement of a given MP. This can be illustrated in Figure 1. A simple statement of the general analytical approach (e.g., atomic absorption) would represent the minimum degree of definition. The application of several analytical constraints upon a general principle (such as sample diultion or calibration by bracketing) would represent increasing degrees of definition in the statement of the MP. This evolutionary process could be expanded to the specification of necessary characteristics of a method in routine use, or to a candidate reference method, and ultimately to a reference method. The Working Group recognized that this broad interpretation would blur the clear distinction between principles and methods. It does, however, reflect the actual situation in which a stated analytical principle, appropriately constrained, could in fact develop from an interim solution into the reference method itself. In any case, it must be realized that the specification of an analytical (procedural) basis and an appropriate set of constraints may place the selected MP for any analyte at any point on the development scale in Figure

1. which is a "continuum" which reflects both the use of the MP and the evolutionary process.

Definitions of Methodologic Principles

Because of the lack of clear definition of the term "methodological principle" as used in the Conference agenda (and elsewhere), after considerable discussion, the Working Group arrived at four "in use" (working) definitions which embody the major concepts. Without prioritizing and without commentary on their appropriateness to the Conference objectives or their ultimate utilization in a national reference system, these topics were covered in a series of "sense resolutions" which are included in this report.

Definition I: An MP is a description of an analytical approach which is considered competent to assign analyte values to assayed reference materials in the absence of a designated consensus reference method.

Definition II: An MP is a fundamental analytical approach selected on the basis of its analytical strength and its potential for development into a reference method.

Definition III: The MP is an analytical concept and/or set of conditions sufficient to characterize a method for routine laboratory use but with sufficient latitude to embody multiple specific methods within its scope.

[MP Definition III recognizes the specific problem of general (language) usage in which a routine laboratory approach will often be characterized by statements very similar to language used to characterize an MP, i.e. "a glucose oxidase method."]

Definition IV: An MP is an analytical approach and set of associated specifications which are used on a provisional basis in the situation where the reference method is not fully developed, or at the current state of the art is not likely to be developed.

Operational Parameters of the Use of MPs in a National Reference System

(a) Consensus: In the context of the National Reference System (NRS) an MP must be derived from a broadly based consensus of the national community. Adequate provision must be made to encourage input from all elements in these stages: (i) initiation of developmental activity; (ii) participation in the development; (iii) review and consensus acceptance; and (iv) reconsideration of existing MPs. The Working Group unanimously endorsed the premise that NCCLS or a like organization must assume responsibility for guidance of the national effort in the area of MPs, and referred the definition of a specific mechanism within this guideline to the Conference Working Group on Organizational Component.

(b) Periodic review: The working group expressed strong support for the absolute need for regular review of any MPs adopted by consensus. It felt that, as a primary goal, a NRS must assure that there is adequate provision to allow for rapid inclusion of the new developments into the system, and that the adoption of any MP would not have an inhibitory effect on further technologic development.

(c) Regulatory aspect: Regulations impact on all elements of the clinical chemistry community, and hence were a major concern of the Working Group. After considerable discussion it was agreed that because of their basic general nature, MPs offer a distinct advantage in the approach to product regulation.

(d) Response time: It appears that MPs can be developed in a relatively short time frame (as illustrated by PSC-5), whereas reference methods often require considerably longer development periods. This is judged to be a distinct advantage of the MP in that it offers a rapid response to a specific need. The NCCLS mechanism for seeking a broad based consensus response seems to work acceptably for this purpose.

(e) Flexibility: The MP concept, with its potential for varying degrees of definition, can readily be adapted to reflect the state of the art. Since the MP is by nature an informal document, it can be updated to reflect changes with a minimum of effort.

(f) Interlaboratory comparisons: The working group recognized that descriptions of methods in conjunction with interlaboratory comparisons and characterizations of methodologies using the methodological principle concept have considerable common ground. The Working Group, however, deferred formal discussion of the ultimate potential role of the MP to a future date, noting that interlaboratory comparison was not a major topic of the Conference.

Criteria and Requirements of Methodologic Principles

The Working Group identified (without priority ranking) a variety of critical/necessary/desirable or possible characteristics of MPs for implementation in the NRS. The priority, or even the relevance of individual criteria and requirements would, of necessity, change as discussion of MPs centered about one of the particular in-use definitions. In addition, the Working Group recognized that the degree of definition as well as the relevance of certain criteria are interrelated. The list of criteria and requirements which must be considered by any consensus body developing a consensus MP includes the following:

(a) General availability of the methodology to the laboratory community.

(b) Transferability of the method among laboratories.

(c) A demonstrated level of precision at appropriate analyte concentrations based on medical needs.

(d) A demonstrated accuracy at an acceptable (medically useful) level, or a known, definable bias, in at least one published method embodying the MP.

(e) The technology on which the MP is based should be the property of no one organization.

(f) There should be a demonstrated need (potential benefit) to developing and adopting the MP.

(g) There should be sufficient scientific data to support the choice of the MP.

(h) Selection of an MP should consider cost effectiveness.

(i) The selected MP should be based on good analytical and biochemical techniques.

(j) Development of an MP should overtly involve all relevent (interested) manufacturers in cases where this is possible (e.g. control serum manufacturers).

(k) Selection of an MP should reflect methods in broad use with sufficient experience; routine use is not a necessary criteria.

(l) Selection of an MP should reflect the natural selection process of the scientific community, accepting guidance from history and the state of the art in practicing laboratories.

(m) The MP should include a statement specifying a minimum of one method in the literature as an example of an actual method which embodies the principle.

(n) When citing a literature method as embodying the MP, consideration should be given to a method with a well-defined or known normal or expected range.

(o) The selection of an MP should be based on the degree to which the limitations on the procedure (i.e. interferences) are known.

(p) All reasonable methods should be considered during the selection process; reasons for non-selection (of common or "in wide use" methods) should be given.

(q) The MP selected should have adequate sensitivity.

(r) The MP selected should avoid specific instruments and technologies.

(s) Ideally, reagent preparation should be simple and reagents should be stable.

(t) The MP should distinguish between use for acute and routine patient care (if MP Definition III is being utilized).

(u) The MP chosen should perform on native human serum as it does on standards or assayed reference materials.

(v) The MP should have available a suitable standard [calibrator].

(w) The MP should not have known bias if an alternative is available.

The "Sense Resolutions":

(1) The Working Group unanimously adopted in-use Definitions I, II, and IV; Industry representative, Dr. Indricksons, was the lone dissenting vote to adopting Definition III. While adopting the four types of in-use definitions as a working basis, the group collectively expressed strong concerns as to the issue of the relative importance of the four classes of MPs. It was agreed that the four in-use definitions covered the broadest scope of possible applications of MPs, especially in the context of implied Conference classification of all methods into classes: definitive methods/ reference methods/ and other types of methods. It was further agreed that pertinent commentary had to address each IN USE DEFINITION to express accurately the Working Group's position on the topic (see below).

(2) (MP Definition I) It was agreed by all members present and by all observers that the NCCLS proposed standard on MP, PSC-5, embodied the concept of an MP included within the scope of this definition. While accepting the concept of PSC-5, and while stating for the record strong favorable support for the document, the approach, and the work-product contained therein, the Working Group stopped short of specific endorsement. The Working Group felt, for example, that it should be possible to specify a triglyceride procedure.

(3) (MP Definition I) The Working Group unanimously

agreed that the MP concept as embodied in PSC-5 is not at variance with the provisional reference principle as outlined in the working documents of this Conference and the CDC position paper. In the context of this national Conference on understanding, the Working Group did not wish to restrict its deliberations to the single conceptual use embodied in MP Definition I, however.

(4) The Working Group unanimously agreed to substitute the term "assayed reference material" to replace the word "calibrator" in the context of its discussions and deliberations. Specific reference was made to the word "calibrator" as used in the PSC-5. There was lack of consensus understanding on the part of the Working Group as to the meaning of the "calibrator," especially as it might be applied to primary standards; "assayed reference materials" was, however, acceptable to the group.

(5) In response to a specific question from the chair, the Working Group agreed that reference serum and controls with assigned values were included in the term "assayed reference materials." There are, in addition, other types of materials or products which are also included in the scope of this definition, but deliberately left unstated at this time. Unanimous consent was achieved on this wording change; reservation as to its need was expressed however.

(6) (MP Definition I) The Working Group expressed unanimous and strong support for the position that every MP should contain a statement citing at least one literature method as an example of a method embodying the MP.

(7) (MP Definition I) Significant concern was expressed by industry, with the strong support of several of the observers, as to the nomenclature used by the Conference in reference to principal assigned values (PAV). This concern addressed the (i) implication of, (ii) ultimate use of, and (iii) application of PAV's to the end use of the product by laboratories in the field. The "PAV" is also used in PSC-5 and in earlier FDA discussions.

The committee took no formal poll on this subject, but deliberated several important points which are included below in the record of the Conference:

Principal Assigned Values

1. There was either agreement or passive non-opposition to the position that the term "principal assigned value" may have an overly strong connotation in the mind of the end laboratory user. It was suggested that an alternative term might be found. The discussion of semantics was ruled out of order by the chair with the notation that the issue would be incorporated in the record. No formal vote was taken.

2. A fundamental use of the "principal assigned value" (PAV) concept is to provide a means of intercomparison (based on analyte values determined by a common MP and recorded in the package insert) between different manufacturers' products. It is recognized that during the debate the group faced an apparent philosophical conflict. A basis of comparison without rigorous definition of and adherence to methodology would be open to considerable quantitative latitude and, therefore, misunderstanding. On the other hand, the group generally recognized the potential value in creating a degree of consistency through general acceptance of an MP for assays of reference materials. This ambivalence was not resolved, although it was reaffirmed that it was a valuable distinction in the development of an MP.

(8) (MP Definition II) The Working Group agreed that development of reference methods is invariably preceded by a selection of an analytical concept or MP on the basis of its potential for development to a sufficient degree of analytical capability to become a reference method. In this respect, the conference areas of reference methods and MP have significant overlap; the Working Group, however, fully recognizes the selection process as embodied in MP Definition II, to be in the purview of reference method development in an NRS.

The group unanimously agreed that selection of an MP under MP Definition I or IV would obviously exert an influence on the ultimate selection of consensus reference methods. The Working Group recognized that the selection of an MP for purposes of Definition II must logically be based on considerations relevant to the desired end use.

(9) (MP Definition III) The Working Group came to unanimous consensus on four criteria: It recognized the importance of promoting the evolution of clinical chemistry and the use of high quality methods in routine clinical laboratories. It recognized that selection of an MP under any of the definitions I, II, or IV would exert an influence on this evolutionary process. It agreed that the traditional categorization of analytical methods by general type, such as "neocuproine", "glucose oxidase", or "hexokinase", resembles very closely the language used to designate MPs. The group concluded that the use of MP definition III is not within the scope of the Conference but is nevertheless an area of high national priority and that it impacts on the broad concept of methodological principles in a unique way.

(10) (MP Definition III) The industry representative (Dr. Indrickson) and several of the observers from industry, did not endorse MP Definition III on the ground that it might prove unduly restrictive to introducing new methods into the laboratory (e.g. glucose dehydrogenase). Industry supported the position of promoting the discontinuance of inferior methodologies by laboratories. The industry representative emphasized to the Working Group that industry's concern was not conceptual but rather mechanistic in nature; the group could not offer a rational mechanism for implementing such a process that did not appear potentially restrictive to the development and introduction of new techniques. After expressing an appreciation for the nature of the concern, the Working Group decided not to work further in this area. It was the sense of the chair that the other delegates were sympathetic to the concern of the industrial representative but voted support of the definition on the grounds of its potential importance.

(11) (MP Definition IV) The group unanimously agreed that for some analytes there is very little likelihood of being able to develop a reference method in the near future. The group unanimously endorsed the concept of using an MP (or provisional reference principle) in lieu of a reference method in these situations, and expressed its unanimous concern that selection of an MP under MP Definition IV must be subject insofar as possible to the development, verification, [transferability], and certification process applicable to the reference method. The group restated its

concern that selection of a provisional reference principle and an MP for some other purpose are separate and distinct processes which should be approached in a manner consistent with the desired end use.

(12) The group took cognizance of the fact that MP Definitions II and IV have a potential for overlap and that both of these activities would logically fall under the purview of "reference method development" in an NRS. The Working Group unanimously agreed that while a potential conflict existed, it only reflected that the area of "methods" represented a continuum of activity in an NRS. The Working Group in its report took cognizance of the importance of the national reference system and its primary leadership role in selection of needs and setting priorities.

(13) Dr. Massion (CAP) introduced a recommendation which in effect would have placed MP Definition I in the status of the primary use of MPs in a national reference system. The recommendation was withdrawn after considerable discussion in which the Working Group expressed a strong support for MP utilization in accord with the recommendation at the present point and time. However, there was not general support for the recommendation, apparently on the basis that the other uses are also matters of great national concern and importance. Several discussants expressed the feeling that MP Definition IV may indeed prove to be the most important MP application. It was the sense of the chair that the resolution was not acceptable to the group on the basis of its desire to remain unrestricted in the development of a conceptual use of MPs in the context of this preliminary and exploratory Conference on a national understanding.

PARTICIPANTS COMMENTS

Dr. Schoen (ASCP): Actually, the ASCP has published its position paper. It was written by Dr. Thorne Butler, and I am essentially substituting for Dr. Butler. I am assuming the prerogative of trying to interpret what the position paper means. The ASCP has been preoccupied by what the clinical laboratories are or might be doing in providing services to patients and the definition of provisional reference methods was not approached in

this way. Probably we are introducing just another categorical aspect to the problem by referring to so-called recommended methods. The ASCP feels that perhaps this group should try to establish some criteria of standards for recommended methods that might be of use within clinical laboratories that are providing services to patients. Recommended methods in essence would be practical methods. There could be more than one recommended method for any given analyte without any real limit to the number of methods that might be classified as recommended if they fit the purpose and criteria of performing throughout the purview of clinical applications and medical usefulness.

There are many approaches to establishing criteria and standards for recommended methods. I would like to comment about the documentation of medically useful information. When it really comes right down to it, the grass roots laboratory, utilizing a working (practical, recommended) method, requires for appropriate application to patient care, some documentation prior to using the method. The documentation needed is information on accuracy, precision, normal values, and diagnostically significant abnormal values. Small specimen volumes applicable to pediatric patients (and patients with inaccessable veins), rapid turnaround time, cost effectiveness and other aspects of service needs should be involved in describing criteria for recommended methods. In essence then, I would like to summarize by saying that the ASCP is not really taking the position to vitiate all of the other definitions of the provisional methods such as methodological principles. I would say that it is in accord with these objectives, but it is also making the plea for considering a category of "recommended practical methods" possibly expressed as multiple recommended methodological principles, in order to bring the advantage of standardization and recommendations directly to the workbench of laboratories providing services to patients. This is the ultimate essence of our objectives to provide improved patient care.

Dr. Tiffany (AACC): AACC found some difficulty in, first of all, defining methodological principles and I think there was some difficulty in handling and accepting the concepts totally because of related questions that we felt first had to be answered. I think our best consensus effort represented in the AACC position paper. We tend to agree with Dr. Dayton Miller of CDC that there is a definite need to define what is meant by methodological principles. When you're trying to outline the position paper, the problem of distinguishing principles versus methods clearly reveals the need for clear definitions. Since several principles are possible for any given analyte, we need to delineate that principle into some sort of transferable type method. As I said, our committee was able, after several sessions, to arrive at the concepts embodied in our position paper. We did establish that there are many important (necessary) analytes for which reference methods are currently unavailable. Provisional methodological principles may be useful as an interim device but it has to be with consensus agreement. We felt that this approach might be useful to give reference points for measuring performance of measuring instruments, reagents, or test kits for manufacturers or for specifying principal assigned values for calibrating materials. Again, we want to emphasize the point that such a consensus is provisional in nature. There are a number of fears (such as those Dr. Thiers addressed this morning), particularly the fear that provisions of methodological principles may be incorporated in the regulatory product class standards for individual analytes. Methodological principles can be a step toward a reference method but also are useful as an interim basis for establishing assigned values for reference materials.

We recognize the importance of the efforts of CDC and CAP in proficiency testing and the various supporting effects of the programs, particularly the excellent educational nature of those programs and the improvement derived from that education. We

encourage this working group to accept (as a consensus group) methodological principles, but to emphasize the educational value as opposed to the regulatory aspect. We have established a set of guidelines which we consider to be the guidelines for establishing methodological principles. AACC considers it of paramount importance to apply the principles which it included in its position paper, especially those quoted below.

(1) Favors the use of sound scientific criteria rather than consensus agreement or balloting for the selection of reference methods and of provisional methodological principles.

(2) Urges that provisional methodological principles be considered a temporary expedient for interim use only, and that such selections not be given undeserved credence by incorporation into product class standards for individual analytes or be made the obligate basis of selection of reference methods.

Dr. Massion (CAP): Well, Dr. Schoen was in better shape than I because at least Dr. Butler wrote a position paper addressing this particular topic. Those of you who have read the CAP position paper realize that there is little mention of provisional reference methods. So, I have to read between the lines and try to interpolate our stance. I think that the CAP would prefer to regard all reference methods as provisional, that everyting methodological is temporary. I have no fault whatsoever with Dr. Miller's outline of provisional reference methods except that he says provisional equals temporary. Temporary also implies eventual permanence. I would hate to see any method so chiseled in stone or so cast in concrete that no provisional reference method again will ever displace it. I think that we feel that reference methods in general should be approached fairly cautiously. We kind of wonder around the edges -- what the purpose of reference methods is. Now that may seem naive, but we've seen in the CAP data the

ability to transfer information directly from definitive methods and the survey serum material, there has been the ability to define, very precisely, the biases between methods. At the present time, I am personally working with the College on an enzyme survey, and I can see that I can determine bias, not only between methods but also between types of instruments and suppliers of reagents and possibly even between lots of reagents. We have a huge number of figures and determinations that come from the surveys and when these are subjected to reasonable statistical analysis for a number of parameters all of them become very interesting. We certainly would wonder about the cost of establishing provisional reference metods that might then become permanent reference methods because if the costs are going to be high, and there certainly is that indication, one has then to consider the benefit to the patient. It doesn't matter who develops reference methods. The costs are still there, and there is in reality only one pocket that they come out of regardless of whether it is called the taxpayer's or the patient's. So, we would want to approach this very cautiously and examine it carefully.

Dr. Indricksons (Industry): The industrial sector within clinical chemistry is the major supplier of reagents and instruments to practicing physicians and consequently has a major interest in methodologies which have the potential of being designated as reference methods or provisional reference principles. A voluntary system for reference methods based upon sound scientific principles and thoroughly evaluated in the practicing clinical environment has merit. However, certain criteria should be considered as the concept of reference methods and their logical precursors, provisional reference methods, are discussed.

1. It should be shown that a significant improvement in patient health care can result from the increased accuracy of a test result. It should be obvious that unless this

fundamental criterion is met, the entire exercise in reference methods is merely an academic endeavor, the cost of which should not be borne by our health care system and ultimately, the patient himself.

2. Since the field of clinical chemistry is rapidly changing, a mechanism should be present to evaluate new methods and replace existing reference procedures by updating methods on a regular basis such as 3 years, or sooner if technological breakthroughs occur. For example, the extensive effort by CDC and associate personnel has demonstrated that the hexokinase method appears to be suited for potential use as the glucose reference method. However, as the glucose dehydrogenase system is developed, it may be possible to obtain results which are as accurate, if not more so, using a much simpler chemical system. Therefore, the very real possibility exists that a reference method will be obsolete by the time its development is completed.

3. The development of a system of reference methods should be entirely voluntary and under the guidance of a body which has the broadest possible representation from all the clinical chemistry profession. For this reason, industry supports the NCCLS, the National Committee for Clinical Laboratory Standards, as the coordinating agency.

4. The development of a system of reference methods and their precursors must be done from a strictly scientific basis separate from possible regulatory uses. We are extremely concerned that once a procedure has received regulatory approval, it would be difficult if not impossible to change it to reflect advancing technology. This is of even more concern in the area of provisional reference methods since by their very definition they have not undergone the close scrutiny of the clinical chemistry

community, and data on accuracy, interfering substances, precision, etc. is incomplete. For these reasons, industry feels that regulation by provisional reference methods is not in the best interest of patient health care and is opposed to their use for this purpose. The entire concept of rigid formal standards for regulatory purposes is more suited to (a discipline with) a mature, stable history rather than a dynamic, rapidly advancing area such as clinical chemistry. It may be worthwhile to consider alternative approaches for judging in vitro diagnostic tests. One such approach might be based on use of parametric type standards rather than reference methods. We seek clarification of the terms, provisional reference principle and provisional reference methods, since they appear to be used interchangeably in some position papers. Therefore, to briefly summarize our position, we support the development of reference methods and their precursors where significant improvement in patient care will result. Two, we are opposed to the regulation of the in vitro diagnostics community by provisional reference methods.

Mr. Alfred Bracey (FDA): It has been previously mentioned that the concept of methodological principles has been converted into reality. I had the distinct pleasure of working with the FDA Diagnostic Products Advisory Committee's Clinical Chemistry Subcommittee at the time the concept was formulated. I further had the challenge of initiating a contract which ultimately led to the development of methodological principles for a limited number of analytes by the NCCLS. One of the major issues at the beginning of the contract was to try to come up with an operational definition of a methodological principle. At that time, the concept of methodological principles consisted of the indentification of a general method and a number of constraints which would vary from one analyte to another depending upon the degree of agreement that could be reached. Dr. Laessig, as most of us know,

served as Chairman of the NCCLS Experts Panel that developed the methodological principles has already been stated. The FDA fully endorses those reasons, particularly those that point to the need of having something to fill the vacuum until reference methods are available. There is an acknowledged need for reference methods. The essential question is, what can served a useful purpose in the absence of reference methods? In the main, the methodological principles are anticipated to have a very valid use. Methodological principles can probably best serve as some type of guideline, sort of pointing in the direction in which reference methods need to go. I think that they will also identify certain methods that may serve as primary candidates for consideration to be developed into a full-fledged, validated reference method, retaining all of the good elements of reference methodologies - those that have been mentioned by previous speakers such as accuracy, precision, reliability, etc. I think the most important contribution of the methodological principles (approach) is that it will represent a first step in the direction of the development of reference methods. Because the time required for the development of fully validated reference methods is relatively long, an interim approach or mechanism is desperately needed. So the concept of methodological principles will certainly serve a valuable need in the meantime while fully characterized reference methods are being developed. At least the scientific community will have the benefit of having some kind of reference bias for analytes in clinical chemistry calibrator in which concentration values are assigned by assay.

Dr. Al Free, NCCLS: I think that the majority of the folks in this room are to some degree familiar with NCCLS in the position paper that the organization submitted as a brief description of the organization which is (1) that it represents a group of industries, professional associations, government agencies, and trade groups, and one simple objective, that is the effort to create standards for clinical laboratory methods, principles, and

products which are based on the collaborative input of all of the various segments of users, producers regulators, and the scientific and medical community.

If there is any single criterion which the organization has tried to use in the definition of what materials or what things are going to be used for the creation of standards, the concept of medical usefulness or utility and clinical relevance have been continuously stated. And certainly, at the level of the nine committees that are charged with overseeing the creative activity in the establishment of standards and in the Board of Directors and Executive Committee, I think this whole concept of utility and relevance is one that gets mentioned again and again.

The evolution of a standard within the framework of the NCCLS is one that is totally and completely based on the concept of peer review and consensus. I'm aware that the word, consensus, is not an easy one for anyone to define, and I sort of suspect that each of us as we have occasion to use it have, to some degree, our own personalized version. But by and large as I would identify consensus, it is a vigorous effort to try to be aware of and incorporate the attitudes of all of the different groups and have a product that does reflect these attitudes.

When Ralph Thiers gave his introductory comments, I was impressed by one of the fears that Ralph mentioned as having been expressed to him which was that the existing structures might be pushed aside which are presently capable of carrying out some particular aspect of this whole program. I, certainly, as a representative of the NCCLS, would emphasize that it seems to me that the NCCLS is a group that has a dedication to the concept of peer review in the areas of the development of standards or the reviewing of standards or the updating of standards or almost any other aspect that you might care to mention. I think it is particularly

appropriate to emphasize again, as has been mentioned already, that a very clearcut example of the sort of thing that the NCCLS does have as a capability is the report that you have here in front of you that relates to the contract that NCCLS entered into with FDA some 2 years or so ago. And I can assure you that from the standpoint of the NCCLS as one of the contractors that there was a very careful and extensive and continued deliberation to make sure that the basic concepts of the NCCLS were respected and incorporated into the contract. I think that I have yet to encounter any attitude within our organization that has been one of displeasure as relates to the final effort that was produced under the chairmanship of Ron Laessig. With the input of my colleagues here, Al Bracey on the left and all of the other people who worked so hard to create this, I would consider it a dramatic example of methodological principles. I think our job is a lot simpler and easier because we have this model as something concrete that we can talk about. It is important to recognize that the NCCLS as a peer review group certainly is not confined to the approach that was used in this particular example but certainly it does exemplify the concept of arriving at an endpoint by the consensus process. I think that pretty well represents the NCCLS message.

COMMENTARY OF INDUSTRIAL REPRESENTATIVES ON RECOMMENDATIONS OF WORKING GROUP 3.3 - PROVISIONAL REFERENCE PRINCIPLES

(Received June 7, 1978)

After consultation and agreement among the industrial representatives to the Conference on a National Understanding for the Development of Reference Materials and Methods for Clinical Chemistry, it is important to clarify industry's position in the Summary Report with reference to Working Group 3.3 - Provisional Reference Principles:

(1) Without taking a position on the use of reference methods for determining principle or primary assigned values, industry takes the position that provisional reference principles should not be used to determine primary assigned values because the methods are too ill-defined.

(2) Though the committee report included assayed control sera in its definition of assayed reference material or calibrators, industry feels that a clear distinction should be drawn between control material used as a calibrator and assayed or unassayed control material used for quality control purposes.

Control material, either assayed or unassayed, when used for quality control purposes is not a calibrator and should not be subject to the requirements of a calibrator. This position is consistent with that of NCCLS Approved Standard ASC-2. According to the distinction between controls and calibrators, primary assigned values should not be applied to controls because control material is not generally intended for comparison between or among products.

FINAL PLENARY SESSION
NOVEMBER 17, 1977
3:00 P.M.

DR. BAYSE:

Our final plenary session will consist of reports from each of the seven working groups including their major consensus recommendations and of closing statements by the sponsors and co-sponsors of the Conference.

One item that has come from the organization working group that I want to address is the possibility of another meeting. We have discussed this with the coordinators and we believe that this is too early to schedule anything of this type. I feel that this question should be determined after the reports of the working groups have been prepared. Probably somewhere in February the Steering Group should get back together and discuss this as a possibility. I think that is as far as we can go with that today. Tomorrow all of the working group chairmen and also some of the Steering Group members will meet at the Center to prepare their written reports. They promised in session last night that they will finish tomorrow and that we will have a mailing next week to the entire conference. That's a very ambitious undertaking and we are going to try to hold to it.

The form for this session will be as follows: Each of the working group chairmen will give a ten minute report. Following this report, members of the Steering Committee (sponsors and co-sponsors) have been allotted four minutes of discussion apiece. If after those two items, the working group report and the discussion of the Steering Committee, there is still time available before 4:30 (when we must close), we will open it up for general comment - two minutes for each participant or observer. (None of the comments today will be recorded for the record unless they are submitted in writing). Of course, the reports of the working group chairmen will be written and any other comments to be part of this record will have to be submitted in writing later, after the conference. So, to get under way with the working group chairmen reports, we will begin with the report from Analytical Goals and Quality Requirement working group, Dr. Frank Elevitch.

DR. FRANKLIN ELEVITCH. Thank you, Dr. Bayse. This working group considers that the primary mission of the National Reference System in Clinical Chemistry is to provide a framework within which true values in clinical chemistry can be sought in order to achieve compatibility from laboratory to laboratory over long periods of time, and with this overall purpose in mind drafted eight recommendations for the Conference. (See Reports).

We feel that these are recognizable propositions that have meaning to many people and should serve as realistic bases for future deliberations. With this work done, the group felt that there was sufficient urgency and effort for considering some practicality after these philosophical deliverations to pass the following resolution for the Conference consideration by a majority vote and I would like to read this resolution at this time.

RESOLUTION

"WHEREAS major portions of a National Reference System in Clinical Chemistry exists today, contributed to by government agencies, private industry, professional organizations, and many individual clinical laboratory professionals, and

Whereas this conference has established goals for further development and implementation of a formal National Reference System for Clinical Chemistry, and

WHEREAS it appears to be desirable to coordinate the relationships and activities of all participating individuals and organizations concerned, and, in order to implement these goals in a timely and orderly fashion, then

BE IT RESOLVED (1) The NCCLS be requested to be the coordinating body to implement the recommendations of the conference on a National Understanding for the Development of Reference Materials and Methods in Clinical Chemistry, and (2) The NCCLS prepare an organizational operating and fiscal plan for this implementation within six months of the present Conference, and, finally, the NCCLS submit this plan to each of the sponsoring organizations and attendees of this current Conference for comment and recommendations."

That is our report, Mr. Chairman.

DR. BAYSE: For the organizational component of the National Reference Systems for Clinical Chemistry - Dr. Boutwell.

DR. JOSEPH BOUTWELL: We came up with eleven recommendations which were passed without dissent. Several failed to pass because of significant dissent. At the beginning of our discussion there was almost total dissent. Instead of reading all eleven recommendations (See Report), I will summarize the more critical ones and I will read one which deals with the overall concept as an outline for a National Reference System. The Conference endorses the concept of improved coordination of the components of the national reference system in clinical chemistry so that they can be more effectively applied. The coordination should be through a voluntary consensus organization in order to insure open decision making, effective communication, identification of needs and development of priorities. The Conference believes that the sponsors and co-sponsors should reaffirm their commitment to the consensus mechanism implemented through NCCLS.

The international implications of a National Reference System were well recognized in our discussions and were additionally emphasized by the presence and the comments of several of our European colleagues, which we greatly appreciate. We also greatly appreciate the freedom and responsibility evidenced in their comments.

DR. BAYSE: For the Standard Materials working group, Dr. George Uriano.

DR. URIANO: The Standard Materials working group members welcome the opportunity to participate in an activity of this nature and would like to see such activities continued in the future. We would hope that the recommendations of our particular group would serve as a basis for formal action over the next few years. I might add that we seem to be in agreement with the previous two speakers in that the NCCLS will obviously be the key for implementing most of these recommendations, as you will see as I go along. All

recommendations in our particular group were in essence achieved by unanimous consent of the group members. (See Reports).

With respect to the establishment of priorities, the Group recommends that NCCLS establish a mechanism to set priorities for the development of new certified reference materials. The prime criteria for setting priorities should be dictated by patient needs. The NCCLS should further establish a panel of experts to help set priorities. The panel would consist of representatives of all segments of the health care community. Priorities should be revised periodically as appropriate.

That's my report, Mr. Chairman.

DR. BAYSE: For the Biological Reference Material working group, Dr. Ray Vanderlinde.

DR. VANDERLINDE: (See Reports). We adopted the ISO definition for certified reference materials. We had a lot of difficulty agreeing on a definition for a biological reference material and instead the name and designation got changed to Biological Validation and Transfer Material (BVTM).

As an example of a typical detailed requirement, referral was made to the NCCLS document on calibrators, ASC-2 standard or its revised version. A formulated protocol for the preparation of each BVTM should be developed. The assignment of target values, the types of validation necessary and testing for stability and homogeneity will be part of the formulation protocol which should be submitted to peer review through the consensus mechanism. The final accreditation and certification should be through an NCCLS-like consensus mechanism.

Labeling - Labeling and certification information should meet all FDA requirements. The group felt very, very strongly that the consensus system should be open to all and, as such, I am happy to hear that the organizational and analytical goals recommended this also.

I would also like to thank the observers and

international visitors to our group who helped us in our deliberations.
Thank you.

DR. BAYSE: For the Definitive Methods Working Group, Dr. Robert Schaffer.

DR. SCHAFFER: In developing our program as we began it we avoided the immediate problem of definitions and, hence, were able to make considerable progress; however, I will take up definition as the first item.

We discussed the techniques that qualify as a definitive method. A great deal of lip service has been given to isotope dilution mass spectrometry (IDMS), but it was recognized in the committee on considering this subject that a number of other techniques provide potential means for obtaining such high quality numbers; i.e., values we expect from definitive methods.

The committee discussed the selection of the definitive method, the characteristics of any such method, and the necessity for its being a method having sound scientific principle; the necessity for specificity; for sensitivity; and the necessity for the method to be used with patient serum (or other biological specimens).

Many laboratories are expected to participate in the development of definitive methods, but, in any event, prior to undertaking to develop a definitive method, the plans for doing so ought to be expressed to the community, and the nature of the techniques to be used, statistical protocols, etc., ought to be given wide publicity in hopes that useful comment would come in benefiting those undertaking that venture.

We recognize the fact that some bias will be found between some definitive methods and the reference method. Now if that bias fits within the expectations and the goals within the bias goals set by the committee, as long as the community has been advised of that, the committee has little problem in presenting the results to the community. Even, if, in fact, the bias found is larger, the committee may wish to reconsider whether these findings are acceptable or not.

This is a very serious problem and the committee would have to deal with this most carefully. Given the fact that a bias may exist, the committee voted to use reference method values as a practical system for the present.

The group's recommendation was to accept the AACC glossary definition for definitive method. In this vote, there was one negative vote and one of the delegates was absent. We expect comments on that from the delegate who provided the negative vote. (See Reports).

DR. BAYSE: For the Reference Methods working group, Dr. Basil Doumas.

DR. DOUMAS: We have agreed on the need for the development of reference methods when inaccuracies have been shown to exist or when patient care is being jeopardized or impaired. That vote was unanimous in the group.

We have agreed on the mechanism for setting priorities for identifying the methods that have to be developed and on the body that will make these decisions.

We have proposed a council - or whatever other name you want to give it - under the umbrella of the NCCLS. The council will consist of representatives of professional organizations, industry, government, and also users. And by "users" we mean clinicians who are using those methods and can identify the problems in their fields. (See Reports).

DR. BAYSE: Thank you, Basil. For the Provisional Reference Principles Working Group, Dr. Ron Laessig.

DR. LAESSIG: Thank you, Dave. (See Reports). Consensus participation is a paramount objective and we have come down with the statement that an NCCLS-like organization is one model which we would refer to the organizational group for consideration. The consensus should be developed, or should be included in the selection, the development, in devising them, in revising and in testing or validating methodological principles.

A second primary concept is periodic and regular review. The coordinating group should be required to carry out this

role, actively not passively. That is, have a strike force or a steering committee assigned a specific problem of thinking about which ones to review, revise, etc. Broad base commentary both for initiation and participation is essential. The committee recognized that this is a nice concept but it is a difficult one to implement and should be worked on.

Regulatory aspects were discussed and, while this is a consistent problem rearing its head, one of the distinct advantages was that a methodological principle is by definition vague and therefore non-regulatory in nature and this was perceived to be an advantage in some cases.

I would like to thank the general chairman for a superb organizational job on behalf of my colleagues. Literally to be a section chairman all you had to do was to get there - everything else was done for you and this is very much appreciated, Dave. You have a great organizational component and staff.
Thank you.

DR. BAYSE: For the American Association for Clinical Chemistry, Dr. Ted Peters.

DR. PETERS: Thank you, Dr. Bayse.

AACC was an early and willing participant in planning this Conference. We believe that data already showed the need for improved interlaboratory coordination in certain areas. Speaking for the other AACC members here as well as myself and our President, Norbert Tietz, who was able to join the latter part of the Conference, we have enjoyed the opportunity to meet and talk with our colleagues who share concern for clinical laboratory chemistry. In many cases we have never had this opportunity before. We also thank Dr. Bayse and his staff at CDC for their fine arrangements and thank Dr. Joe Boutwell who I know was behind the inception of this Conference. The recommendations of AACC are generally in line with those of several of the Working Groups. First, we believe it is worthwhile to formalize a national reference system for clinical chemistry, although we would start the words with lower case rather than capitals. This system should be an extension of the existing efforts of diverse groups, but with the new feature of unified

coordination at the national level in order to avoid duplication and to provide authenticity. Specifically, we concur with the assignment of the role of the coordinating body to NCCLS as an existing consensus organization, and would mandate it to expand and modify its structure as necessary to assume these following functions:

1. To ascertain the need for improved methodology and for new reference materials.

2. To set priorities for their development.

3. To set the specifications for the reference materials and the goals for the reference methods.

4. To supervise the certification of reference materials and to give official validation to reference methods.

5. To act as a repository for the accumulated data that accrue in this process.

6. To maintain the continuity of the methods, to assure supplies of the reference materials and to provide for periodic revision of specifications and procedures.

NCCLS should perform this task by its consensus process, with open communication and with frequent publication. AACC believes that this approach of coordination of existing diverse resources offers a reasonable and a cost-effective approach to improved health care.

I would like finally to add that I have a fear that we are merely handing NCCLS all of our problems, which may be a Herculean task for it to overcome. NCCLS is going to need our support in the days to come.

DR. BAYSE: For the American Society of Clinical Pathologists, Dr. Thorne Butler.

DR. BUTLER: Thank you very much, Dr. Bayse. I would like to add to what the other speakers have said, that the success of this meeting, which I think has been successful, is primarily due to the efforts of yourself and your staff in organizing a well thought out program that has accomplished a

great deal.

The ASCP position on the development of a national reference system in clinical chemistry embodies the following concepts. One, a method for discovery and recognition of medical needs as pertain to clinical chemistry, and of the setting of priorities for resolutions of those needs. Two, the development of an organization to interdigitate the above priorities with appropriate development of materials and methods and implementation of those recommendations. And three, there should be a feedback mechanism to evaluate the validity of levels established for the priority analytes.

This Conference has done much to support the ASCP's position by generating a better understanding of the critical elements needed for establishment of a national reference system. Many gray areas which needed either definition or clarification, particularly with respect to materials and methods, were basically resolved by the Conference. The ASCP believes that the Conference has taken the necessary preliminary steps for building the foundation for a national reference system based on voluntary participation by organizations responsible for clinical chemistry services.

DR. BAYSE: Thank you. For the College of American Pathologists, Dr. John Batsakis.

DR. BATSAKIS: Thank you, Dr. Bayse. The College of American Pathologists (C.A.P.) has been pleased and privileged to co-sponsor and to actively participate with other scientists in this Conference. C.A.P. further considers that a system for such development is extant and flourishing. It is a process which has evolved concurrently with advances in all aspects of clinical chemistry; not the least of which being the assessment provided by large interlaboratory comparison programs such as those in the Surveys of the College of American Pathologists. The existing system now provides the highest over-all quality in the world. This pre-eminence has been achieved through the cooperative union of clinical pathologists, clinical chemists and other laboratory scientists, be they in practice, industry, academe, or government. A large measure of the success is due to a dedication toward the ultimate analytical goal of clinical relevance, rather than the more narrowly

defined goal of the analysis itself.

These achievements clearly indicate the existing system cannot be condemned as a whole and C.A.P. is of the firm conviction that any apparent weaknesses in this system require definition before changes are advocated. Since we deal with an analytical system, these weaknesses must be defined in analytical terms rather than by an a prior subjective judgement.

To that end, the College of American Pathologists views as the outcome of the conference, the following:

1. An acceptance that scientific application of Survey statistics will be required to define problem areas.

2. A strengthening of the existing process of reference materials and methods so that it may be applied to definable, statistically characterized areas requiring improvement.

3. Selective and judicious application of definitive and "reference" methods to improve ambiguous clinical laboratory information.

4. All changes to be predicted on the ultimate analytical goal of patient care enhancement; not abstract method enhancement.

5. A hierarchical system of materials and methods should not be applied except where need is defined.

6. A consensus body, composed of all contributors to that analytical system be charged with establishing priorities and providing general guidance to the enhancement of the system. The NCCLS appears to be such a body.

DR. BAYSE: For CDC, Dr. Jane Neese.

DR. NEESE:

1. The Center for Disease Control (CDC) supports the development and implementation of a voluntary national reference system for clinical chemistry (NRSCC).

2. The CDC supports the premise that accuracy is the cornerstone upon which the NRSCC is based.

3. The CDC supports the concept that the NRSCC requires a series of interrelated technical components, each having specific uses and performance characteristics, and that no one methodological or material component will meet all of the needs of the NRSCC.

4. The CDC believes that the NRSCC should not rely solely on government agencies to carry out all development, validation and transferability testing. Our concept, therefore, also includes the carrying out of these functions by private initiatives as an essential element in dynamic interaction at all stages, and since the elements of an NRSCC must be widely accepted if they are to be effectively applied, their development and recognition require a national consensus and approval mechanism.

5. The CDC believes that some of the functioning components of an NRSCC already exist. These consist of many interested individuals and groups participating in a series of loosely defined and insufficiently understood processes. These efforts have developed beyond the stage of infancy and have reached a stage in which wider understanding and guidance must be applied in order to avoid conflicting or competing efforts which slow progress.

6. CDC Participants at this Conference believe that considerable progress has been made at these meetings toward developing an understanding of the component processes which are involved in developing, accepting and utilizing reference methods and materials in a national reference system for clinical chemistry.

   For example, the CDC representative in Group 2.1 feels that very constructive progress has been made in establishing an understanding, primarily in the areas of nomenclature, development of specific criteria, and the establishment of a possible mechanism for the certification of reference materials. And the CDC representative in Group 3.3 believes that considerable

progress has been made toward defining terms and in coming to some understanding and consensus on the use of methodological principles (MPs).

7. The CDC looks forward to continuing work in support of and through a national reference system in clinical chemistry.

DR. BAYSE: Thank you, Jane. For the Food and Drug Administration, Dr. Eloise Eavenson.

DR. ELOISE EAVENSON: We from the FDA who have participated in the development of the program for this meeting, in the preparation of a position paper and in the give and take of the various working groups, are very pleased with the steps taken to reach an understanding of the various interests of the various groups represented here and thus to get a step closer to a meaningful National Reference System. We agree that since NCCLS includes all of the sponsoring organizations of this Conference as members, that it is appropriate to ask NCCLS to develop a plan to implement and coordinate the recommendations of the various working groups. FDA intends to cooperate in any way we can to achieve the objectives set out at this Conference.

I have heard expressed in several of the groups a fear that the major thrust of a National Reference System will be to provide FDA with a tool for regulation, and somehow that appears to be bad. Although I can't agree with you who have that viewpoint, I do understand your concern. I wish I could alleviate that concern. Instead, I will try to express my own concern. My concern is that if we don't develop a National Reference System together in a responsible and objective way that can serve as the basis for evaluating performance, some other system may emerge without your valuable input. I will remind you of a couple of things. Congress makes the laws. FDA has been given the responsibility, under the Medical Device Amendments to the Food, Drug, and Cosmetic Act approved and passed by Congress in 1976, to assure that the products manufacturered for use in the clinical laboratory are safe and effective for their intended use. Early in our deliberations with your peers who were members of our Diagnostic Products Advisory Committee it was agreed that the most reasonable way to assure

effectiveness of clinical chemistry products was to assure adequacy and reliability of results obtained by their use. In order to measure the quality of results fairly and equitably and to provide the manufacturer and laboratory user with the same methods used by FDA, reference materials and reference methods established by scientifically sound procedures coupled with the analytical goals required in their use were believed necessary.

A considerable amount of time, money and effort is being expended by FDA and others in order to assure that reference methods and materials used for regulation are developed and applied in an open and scientifically valid way. Although the model of a regulatory standard most often cited is the Glucose Product Class Standard, we have not concluded that the approach to product standards exemplified by the proposed glucose standard is the only one possible or appropriate. Furthermore, we have not concluded that having reference materials or methods for a particular analyte automatically subjects the products to a standard which incorporates those methods or materials. Having a National Reference System, whatever it turns out to be, actively supported and applied by all of us and the organizations we represent, could obviate in some cases the need for the kinds of Product standards considered appropriate a few years ago.

Even if some of your worst fears are realized and FDA does somehow apply the system in its regulations, wouldn't you rather have regulations based on a system you helped to develop?

In conclusion, it is not possible for me to guess whether or not the several components which may evolve from a National Reference System will be used for regulatory purposes in the future. I would urge you not to let that consideration be a primary influence on your participation in the continuing development and implementation of the system. We are all concerned with the improvement of laboratory medicine. We from FDA believe that a National Reference System may be a step in that direction.

DR. BAYSE: For Industry, we have Mr. Jerry Gallwas.

MR. GALLWAS: On behalf of members from Industry, we

appreciate this opportunity to participate in a Conference of such open character.

The precedent this Conference has provided will endure even the most severe disagreements and we will certainly support further such efforts.

While the proceedings were sometimes slow and painful, it was never guaranteed that the birth or rebirth--depending on your point of view--would be an easy matter.

The members of industry can generally support the recommendations you have just heard from the working group chairman. We will reserve our final comments until we have had a chance to review the written word--but, the spirit is right! We were particularly pleased with the coupling of clinically relevant needs to the concept of cost benefit which became a point of considerable discussion in many of the working groups.

Only in one area do we have an immediate concern. That concern is with the recommendations from the Methodological Principles Working Group concerning the use of Methodological Principles for routine laboratory use. While we agree that one of the criteria for a Methodological Principle be the ability to use it in a routine laboratory environment, we do not support the concept of recommending it for routine use. This would tend to polarize the user and regulatory community toward that Particular Methodological Principle thereby tending to limit new thinking and innovation in the development of new methods.

We are pleased with the concept of using the consensus environment--in this case, NCCLS, as the medium for carrying out the activities of the National Reference System. It is important to recognize, however, that NCCLS can only succeed with the commitment from each one of us, both individually and organizationally, all of whom have a vested interest in the improvement of laboratory medicine.

Lastly, we from industry and I particularly, want to thank Dr. Bayse and his staff for their outstanding job in meeting management.

DR. BAYSE: For the National Bureau of Standards, Dr. Phil LaFleur.

DR. LAFLEUR: I would like to echo the comments of our colleagues on the pleasure that we of the Bureau of Standards have had for being able to be here and participate in this Conference.

NBS has been involved in the subject of analytical chemistry and the production of standard reference materials for about 75 years although for only about 10 of those years have we had strong involvement in the health field. We support the establishment of a National Reference System for Clinical Chemistry and the coordination of that effort by the National Committee for Clinical Laboratory Standards.

I, too, would like to compliment Dr. Bayse and the members of his staff on the excellent physical arrangements and the way they were handled.

DR. BAYSE: For the National Committee for Laboratory Standards, Dr. Pierre Keitges.

DR. KEITGES: The President of the NCCLS, Dr. Nadeau, wished to be here to address you this afternoon. He was called away on urgent matters at noon and asked me if I would give the NCCLS statement.

The NCCLS wishes to acknowledge the excellent preparation for the conducting of this national understanding Conference. Individual credits are too numerous to list. The CDC personnel collectively, and especially Dr. Bayse and Dr. Boutwell, deserve special recognition for their efforts.

The NCCLS wishes especially to acknowledge the special cooperation of the CDC in assisting us with arrangements for our fall membership meeting that immediately preceded this Conference.

The NCCLS recognizes the theme of this Conference, that of a national understanding; and we suggest that great strides have been taken in this direction within the past two days. We understand that a number of recommendations in the working groups have expressed confidence that the NCCLS can

and should accept certain significant responsibilities within this national reference system. One of our major charges in light of the recommendations is to act promptly and responsibly to comment on the proceedings of these working groups. This will be done so that the country and others can assess for themselves the interest, resources, and needs of the NCCLS in contributing to the national reference system.

The NCCLS recognizes certain vital characteristics in the national reference system. These characteristics have to reflect both realities and practicalities. Such realities and practicalities are: 1) the NRS should be viewed as a process, a process in which all can contribute and all have a voice in shaping the outcome. 2) The NRS process may involve new relationships between organizations in terms of sharing their resources and interests with a wider range of their colleagues. 3) The NRS process would not seem to involve any major dislocation in the functions of its supporters in their development of reference methods and materials, since after all, in reality, this audience is a significant part of the NCCLS.

The NRS will provide a focus for scientific efforts and should act to conserve on the resources of its supporters. Due to the current extensive debate on health care costs, this facet could be significant.

The NRS process brings extraordinary resources to bear on the interests of patient care and public health in the application of clinical laboratory medicine. Successful pursuit of the NRS process will enable governmental functions to be assisted in determining where the greatest need and opportunities for improvement lie.

The NRS process is truly an awesome challenge. However, it is one that, for its part, the NCCLS will dedicate prompt and eager support and devotion.

DR. BAYSE: That concludes the reports of the sponsors. I will now open the floor to any further comment by participants or observers.

DR. BRADLEY COPELAND: The first point - my initial question to the working steering committee that the rights of

the individual be protected with respect to factual data recommendations and conclusions of the committee, I feel has been wonderfully answered in the discussion groups. I feel that rights of the individual have been completely protected and I would expect that in the publication of the proceedings that this would be the case as well. I thank you very much for your help and appreciate it. Secondly, I would like to comment relative to the use of the term "S.I. Units" in the report of the Standards Material Working Group. The term S.I. Units includes both S.I. Mass Units and S.I. Modlecular Units; the term Conventional Units when used to convey the meaning S.I. Mass Unit obscures the fact that both mass and molecular composition are measurable properties recognized by the Bureau Internationale des Poids et Mesures (BIPM), which is the recognized international body responsible for the metric system known as System Internationale, S.I."

Thank you.

DR. BAYSE: Anyone else? No one else would like to speak? I can't believe it! All right! I would like to thank our international guests - I believe that in the future your participation will bear fruit in bettering our relations with the rest of the world in the development of further systems of reference and clinical practice. I thank you for your attention and the meeting is adjourned.

REGISTRATION FORM

Conference on a National Understanding
for the Development of
Reference Materials and Methods
for Clinical Chemistry

November 16-17, 1977

Return to:
Center for Disease Control
Attn: David D. Bayse, Ph.D.
Director, Clinical Chemistry Division
Atlanta, Georgia 30333

Please enroll me for the Conference_____.

Enclosed is my registration fee ($55.00)_____. *

Choice of Working Group: (1) _____

(2)_____

(3) _____

Name_____

Title _____

Organization _____

Address _____

_____

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Telephone: Area Code ( ) _____

*Attendance at Working Groups will be contingent upon payment of Registration Fee.

Make checks payable to: NATIONAL REFERENCE CONFERENCE

CONFERENCE ON A NATIONAL UNDERSTANDING FOR THE DEVELOPMENT OF REFERENCE MATERIALS AND METHODS FOR CLINICAL CHEMISTRY

NOVEMBER 16 – 17, 1977
HYATT REGENCY HOTEL
ATLANTA, GEORGIA

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Public Health Service

Center for Disease Control

GENERAL FORMAT

The Conference is planned as a two-day meeting for approximately 150-200 participants representing the concerned Federal Agencies, clinical laboratory professions, and the appropriate health industries.

The Conference will address three major topics, each of which will be subdivided into more manageable working groups. Sponsors and co-sponsors will have a designated representative on each Working Group. The purpose of these Working Groups will be to review the scientific advice contained in position papers submitted prior to the Conference by the sponsors and co-sponsors. This advice will be reviewed, and, together with the deliberations of the Working Group, will be organized into the proceedings of the Conference which will be published at an early date.

OBJECTIVES

This Conference will attempt to further the understanding of the components necessary for the development, the acceptance, and the utilization of reference materials and methods in clinical chemistry.

Such a scheme on a national basis can be termed a National Reference System in Clinical Chemistry (NRSCC).

The objectives of this two-day Conference are (1) to define criteria for and essential characteristics of reference methods and materials used in establishing national standards for the accuracy of quantitative methods in clinical chemistry, and (2) to develop a total framework in which government agencies, professional organizations, and industry can cooperate on a national basis in the orderly development of a National Reference System in Clinical Chemistry.

PROGRAM

NOVEMBER 16 (WEDNESDAY)

8:00 – 8:30	Registration
8:30 – 8:40	Announcements (Dr. David D. Bayse)
8:40 – 8:55	Welcoming Address (Dr. William Foege)
8:55 – 9:25	Keynote Address (Dr. Morton K. Schwartz)
9:25 – 10:30	Convene with Topic Coordinators
10:30 – 10:50	Coffee
10:50 – 12:00	Convene Working Group Sessions
12:00 – 1:00	Working Lunch
1:00 – 3:00	Working Group Sessions
3:00 – 3:20	Coffee
3:20 – 5:30	Working Group Sessions
6:30 – 7:30	Mixer
7:30	Dinner

NOVEMBER 17 (THURSDAY)

8:30 – 10:00	Working Group Sessions
10:00 – 10:30	Coffee
10:30 – 12:00	Working Group Sessions
12:00 – 1:00	Working Lunch
1:00 – 2:30	Working Group Sessions
2:30 – 2:45	Coffee
2:45 – 4:30	Working Groups Report to Conference
4:30	Adjournment

CONFERENCE CHAIRMAN: DR. DAVID D. BAYSE, CDC

TOPIC 1. ORGANIZATIONAL DEVELOPMENT OF A NATIONAL REFERENCE SYSTEM FOR CLINICAL CHEMISTRY (NRSCC)

Coordinator: Dr. Eloise Eavenson, FDA

Group 1.1 **Analytical Goals and Quality Requirements Working Group**

Chairman: Dr. Franklin Elevitch, CAP

CDC	Dr. Alan Mather
FDA	Henry T. Lee, Jr.

NBS	Dr. Philip D. LaFleur
NCCLS	Dr. Robert W. Pritchard
AACC	Dr. George N. Bowers, Jr.
ASCP	Dr. Desmond Burke
CAP	Dr. Roy N. Barnett
INDUSTRY	Dr. Stanley Bauer

The Analytical Goals and Quality Requirements Working Group will attempt to identify those laboratory applications requiring specific performance levels. In addition it will evolve a framework for decision making that can serve as a basis for defining such goals in terms of medical usefulness in the development of reference methods and materials. Such a framework could provide a bridge between routine clinical laboratory practice and a national reference system.

Group 1.2 Organizational Component of National Reference System for Clinical Chemistry

Chairman:	Dr. Joseph H. Boutwell, Jr., CDC
CDC	Dr. Gerald R. Cooper
FDA	Dr. Dale Fisher
NBS	J. Paul Cali
NCCLS	Jaxon A. White, Jr.
AACC	Dr. Nathan Gochman
ASCP	Dr. Rex B. Conn, Jr.
CAP	Dr. Roger K. Gilbert
INDUSTRY	William T. Ryan

Organizational Component of National Reference System for Clinical Chemistry Working Group will design an organizational plan that will effectively and efficiently use the available national resources of both government and private sectors. One aspect will be improvement of communications between the professions and promotion of group scientific investigation.

TOPIC 2. GUIDELINES FOR THE TECHNICAL DEVELOPMENT OF THE MATERIALS COMPONENT OF THE NRSCC

Coordinator: Dr. Thorne J. Butler, ASCP

Group 2.1 Standard Materials Working Group

Chairman:	Dr. George Uriano, NBS
CDC	Dr. Richard J. Carter
FDA	Dr. Nabeeh Mourad
NCCLS	Dr. Theodore Peters, Jr.
AACC	Dr. Royden N. Rand
ASCP	Dr. John A. Lott
CAP	Dr. Daniel J. Hanson
INDUSTRY	Dr. Thomas Adams

Standard Materials Working Group will outline and partially specify the criteria for selection, validation, characterizing, and labeling of such materials and will additionally consider priority needs for developing specific standards.

Group 2.2 Biological Reference Materials Working Group

Chairman:	Dr. Raymond E. Vanderlinde, AACC
CDC	Dr. Jane W. Neese
FDA	Dr. Amiran Daniel
NBS	Dr. Dennis Reeder
NCCLS	Dr. Huey V. Auger
AACC	Dr. Robert Rej
ASCP	Dr. Hermann Peter Lehmann
CAP	Dr. John G. Batsakis
INDUSTRY	Dr. Joseph L. Giegel

Biological Reference Materials Working Group will outline and partially specify the criteria for selection, validation, characterizing, and labeling sample-simulating biological matrix materials.

TOPIC 3. GUIDELINES FOR THE TECHNICAL DEVELOPMENT OF THE METHODOLOGICAL COMPONENT OF A NRSCC

Coordinator: Gerald E. Gallwas, INDUSTRY

Group 3.1 Definitive Methods Working Group

Chairman:	Dr. Robert Schaffer, NBS

CDC	Dr. John A. Liddle
FDA	Dr. Charles Furfine
NCCLS	Dr. Matthew M. Patton
AACC	Dr. Ralph E. Thiers
ASCP	Dr. Robert C. Rock
CAP	Dr. John B. Fuller
INDUSTRY	Walter Slavin

Definitive Methods Working Group will outline and partially specify the criteria for selection, validation, and adoption of definitive methods as well as how they will be applied.

Group 3.2 **Reference Methods Working Group**

Chairman: Dr. Basil Doumas, AACC

CDC	Dr. Carl A. Burtis
FDA	Dr. Barbara Tejeda
NBS	Dr. Rance Velapoldi
NCCLS	Col. Edward C. Knoblock
AACC	Dr. Graham Widdowson
ASCP	Dr. Bradley E. Copeland
CAP	Dr. Noel Lawson
INDUSTRY	Dr. Charles C. Allain

Reference Methods Working Group will outline and partially specify the criteria for selection, validation, and adoption of reference methods. This discussion will include reference methods with and without a *definitive* accuracy base.

Group 3.3 **Provisional Reference Principles Working Group**

Chairman: Dr. Ronald H. Laessig, NCCLS

CDC	Dr. Dayton T. Miller
FDA	Alfred Bracey
NCCLS	Dr. David M. Jeffers
AACC	Dr. Thomas O. Tiffany
ASCP	Dr. Thorne J. Butler
CAP	Dr. Charles G. Massion
INDUSTRY	Dr. Andris Indriksons

Provisional Reference Principles Working Group will outline and partially specify the criteria for selection of analytical principles or materials for interim use as common bases of reference.

GENERAL INFORMATION

Attendance will be limited to invitees only. For information concerning attendance, please contact the appropriate representative of the following organizations:

AACC	Dr. Theodore Peters
ASCP	Dr. Thorne J. Butler
CAP	Dr. Roger K. Gilbert
CDC	Dr. David D. Bayse
FDA	Dr. Eloise Eavenson
INDUSTRY	Gerald E. Gallwas
NBS	Dr. Philip D. LaFleur
NCCLS	Dr. Pierre Keitges

Admission to each Working Group session will be restricted to those wearing specific badges for that Group. In addition to participants, a limited number of observers, including international visitors, have been invited.

Advance registration is requested using the form attached to this notice. Please be sure to indicate first, second, and third choice for Working Group attendance.

A registration fee of $55.00 will be required.

Registration should be made on or before October 1, 1977. Registration fee cannot be refunded after this date. Checks should be made payable to the NATIONAL REFERENCE CONFERENCE.

HOTEL INFORMATION

All Conference activities, as well as lunches and lodging, will be at the Hyatt Regency Hotel located in downtown Atlanta. A block of rooms has been reserved by the Conference, but each participant must check in personally at the hotel desk, and is responsible for all hotel charges. Participants and observers should complete the enclosed card in order to obtain the special Conference rate and return it directly to the hotel. Requests for hotel reservations should be made before October 1. Transportation by limousine or taxi is available between the airport and the hotel.

PREPARATION OF CONFERENCE PROCEEDINGS:

On Friday, November 18, a post-Conference discussion will be held at the Center for Disease Control consisting of Topic Coordinators, Working Group Chairmen, and other designated parties to assist in the preparation of the Conference proceedings.